A VALIDATED ESTIMATION OF IBUPROFEN AND FAMOTIDINE IN PURE AND IN DOSAGE FORM BY UV SPECTROPHOTOMETRY AND RP-HPLC

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MASTER OF PHARMACY
(Pharmaceutical Analysis)

Submitted by

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(Accredited By "NAAC" with a CGPA of 2.74 on a four point scale at "B"-Grade)

Melmaruvathur – 603 319.

MAY 2012

CERTIFICATE

This is to certify that the research work entitled **A VALIDATED ESTIMATION OF IBUPROFEN AND FAMOTIDINE IN PURE AND IN DOSAGE FORM BY UV-SPECTROPHOTOMETRY AND RP – HPLC** submitted to The Tamil Nadu Dr.M.G.R. Medical University in partial fulfilment for the award of the Degree of the MASTER OF PHARMACY (Pharmaceutical Analysis) was carried out by **NETHAJI A (REG.NO. 26106127)** in the Department of Pharmaceutical Analysis under my direct guidance and supervision during the academic year 2011-12.

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Date :

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CERTIFICATE

This is to certify that the dissertation entitled **A VALIDATED ESTIMATION OF IBUPROFEN AND FAMOTIDINE IN PURE AND IN DOSAGE FORM BY UV-SPECTROPHOTOMETRY AND RP – HPLC**is the bonafide research work carried out by**NETHAJI A (REG.NO. 26106127)** in the Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr.M.G.R. Medical University under the guidance of **Dr.D.NAGAVALLIM.Pharm.,Ph.D.,** Department of Pharmaceutical Analysis, Adhiparasakthi College of Pharmacy, during the academic year 2011-2012.

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Above all I dedicate myself and my work to **Almighty**, who is the source of knowledge and for showering all his blessings and grace upon me.

NETHAJI A

DEDICATED

TO

MY

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LIST OF ABBREVIATIONS USED

ICH	-	International Conference on Harmonisation
λ	-	Lambda
LOD	-	Limit of Detection
LOQ	-	Limit of Quantitation
mcg/mL	-	Microgram Per Millilitre
mg/tab	-	Milligram Per tablet
mL	-	Millilitre
mM	-	Milli Mole
nm	-	Nanometer
pH	-	Negative Logarithm of Hydrogen Ion
%	-	Percentage
% RSD	-	Percentage Relative Standard Deviation
RP-HPLC	-	Reverse Phase -High Performance Liquid Chromatography
Rt	-	Retention Time
S.D	-	Standard Deviation
S.E	-	Standard Error
0.1 M NaOH	-	0.1 Molar Sodium Hydroxide
UV-VIS	-	Ultraviolet - Visible
USP	-	United States Pharmacopoeia
IR	-	Infra Red
AUC	-	Area Under Curve
°C	-	Degree Celsius
Gms	-	Grams
µL	-	Microlitre
µ	-	Micron
v/v	-	Volume/Volume
min	-	Minute
mL/min	-	Millilitre/Minute

INTRODUCTION

1. INTRODUCTION

1.1 Analytical Chemistry

Analytical chemistry may be defined as the science and art of determining the composition of materials in terms of the elements or compounds in them. It is concerned with the chemical characterization of matter both quantitative and qualitative.

The substance may be a single compound or a mixture of compounds and may be in the form a tablet, pill, capsule, ampoule, liquid, mixture or an ointment.Qualitative analysis reveals the chemical identity of the species in the sample. Quantitative analysis establishes the relative amount of one or more of these species.

Most manufacturing industry rely on both qualitative and quantitative analysis to ensure that the raw material used meet certain specifications and also to check the quality of the final product.

Any type of analysis involves two steps:

Identification (qualitative)

Estimation (quantitative)

In qualitative analysis, a reaction is performed in such a way as to indicate the formation of a precipitate, a change of a colour, the dissolution of a precipitate / complex formation and the evaluation of a gas.

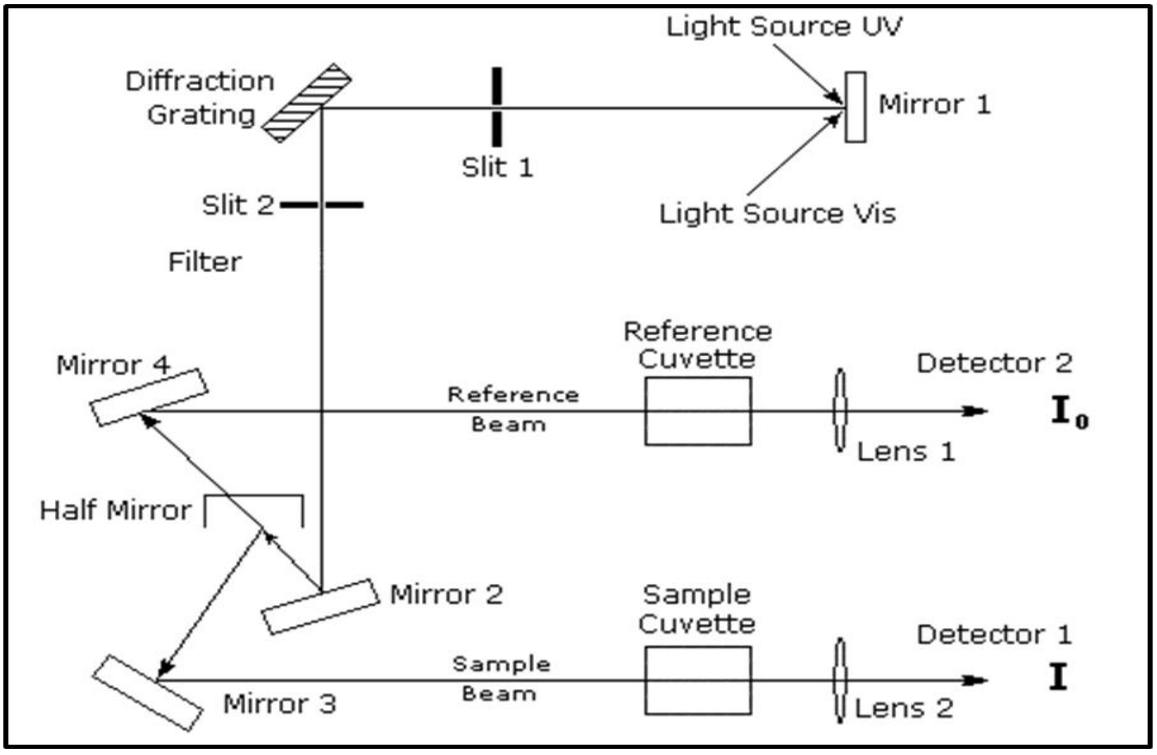
Quantitative analysis is performed ordinarily through five steps. They are sampling, dissolution, precipitation, measurement and calculation.

1.2 Analytical Method Development

Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. The official test methods that results from these process are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products.

1.3 UV – SPECTROSCOPY

It involves the measurement of amount of ultra-violet radiation absorbed by a substance in the solution. The wavelength between 190-390 nm (practically 200-400 nm) is considered to be UV radiations/ region. Colored compounds absorb in visible range i.e. 400-800 nm.



The assay of an absorbing substance can be carried out by using

a) Standard absorptivity Value.
b) Use of calibration graph.
c) Single point standardisation.

a) Standard absorptivity value:

This procedure is adopted by official compendia for the stable substance that have reasonably broad absorption bands and which are practically unaffected by variation of instrumental parameters. The use of standard A (1%, 1cm) value avoids the need to prepare a standard solution of the reference substance in order to determine its absorptivity.

b) Use of calibration graph:

In this procedure, the absorbance of a number (typically 4-6) of standard solution of the reference substance at concentrations encompassing sample concentration are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as a concentration corresponding to absorbance of the solution .

c) Single point standardization

This procedure involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The standard and sample solution are prepared in a similar manner, ideally the concentration of standard solution should be close to that sample solution. The concentration of the substance in the sample is calculated using.

$$C_{test} = \frac{A_{test} \times C_{standard}}{A_{standard}}$$

Where C_{test} and $C_{standard}$ are the concentrations in the sample and standard solutions and A_{test} and $A_{standard}$ are the absorbances of sample and standard solutions respectively.

The use of UV and visible spectroscopy for quantitative analysis employs the method of comparing the absorbance of standards and samples at a selected wavelength. The analysis of mixtures of two or more components is facilitated by activity of absorbance. Other applications include measurement of absorption of complexes to establish their composition. All chromogenic compounds are not suitable for quantitative measurements, i.e. the choice of the system and procedure depends largely on the chemistry of the species to be determined.

d) Points to be considered in the selection of procedure include:

- Stability of absorbance with respect to time, variation of pH, ionic strength and temperature.
- Degree of selectivity of complexing agent includes the effect of other species likely to be present.
- Conformity to the Beer-Lambert's Law and plot calibration data for the range of concentration measured.

1.3.1 Methods carried out:

a) AREA UNDER THE CURVE AND SIMULTANEOUS EQUATION METHOD

b) DERIVATIVE SPECTROSCOPY METHOD

a) Area under the curve method

The area under curve method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength area is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. In combination drugs λ_1 and λ_2 denotes the wavelength ranges of the components. The integrated value of absorbance in the wavelength ranges of both the drugs are substituted in the simultaneous equation to get the concentration of the drugs.

$$c_x = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \text{ and } c_y = \frac{A_1 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}}$$

b) Derivative spectroscopic method

This method involves the conversion of the normal spectrum into first, second or higher derivative spectrum. The transformation that occurs in the derivative spectrum are understood by reference to a Gaussian band which represents an ideal absorption band.

The first derivative (D^1) spectra is a plot of the ratio of change of absorbance with wavelength against wavelength, i.e a plot of slope of the fundamental spectrum against wavelength or a plot of $dA/d\lambda$ Vs λ. At $\lambda 2$ and $\lambda 4$, the maximum positive and maximum negative slope respectively in the D°.

Spectrums correspond with maximum and minimum respectively in the D1spectrum. The λ_{max} at λ_3 is awavelength of zero slope and gives $dA/d\lambda$ 0,i.e a cross-over point, in the DI spectrum.

The first order derivative spectrum of absorption band is characterized by a maximum, a minimum and a cross-over at a $\lambda_{max}$of the absorption band. These spectral transformations confer two main advantages on derivative spectrophotometry. Firstly an even order spectrum is of narrower spectral band width than its fundamental spectrum.

Derivative spectrum shows better resolution of overlapping bands than the fundamental spectrum and may permit the accurate determination of $\lambda_{max}$of the individual bands. Secondly, derivative spectroscopy discriminates in favours of the substances of narrow spectral bandwidth against broad band width substances.

1.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography is convenient separation technique used for wide types of samples, exceptional resolving power, speed and nano molecular detection levels. It is presently used in pharmaceuticals research and development.

1.4.1 PRINCIPLE OF SEPARATION IN HPLC

The principle of separation is normal phase mode and reverse phase mode is adsorption, when mixture of components are introduced in to a HPLC column; they travel according to their relative affinities towards the stationary phase. The Components, which have more affinity towards the absorbent, travel slower. The components, which

have less affinity towards the stationary phase, travel faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

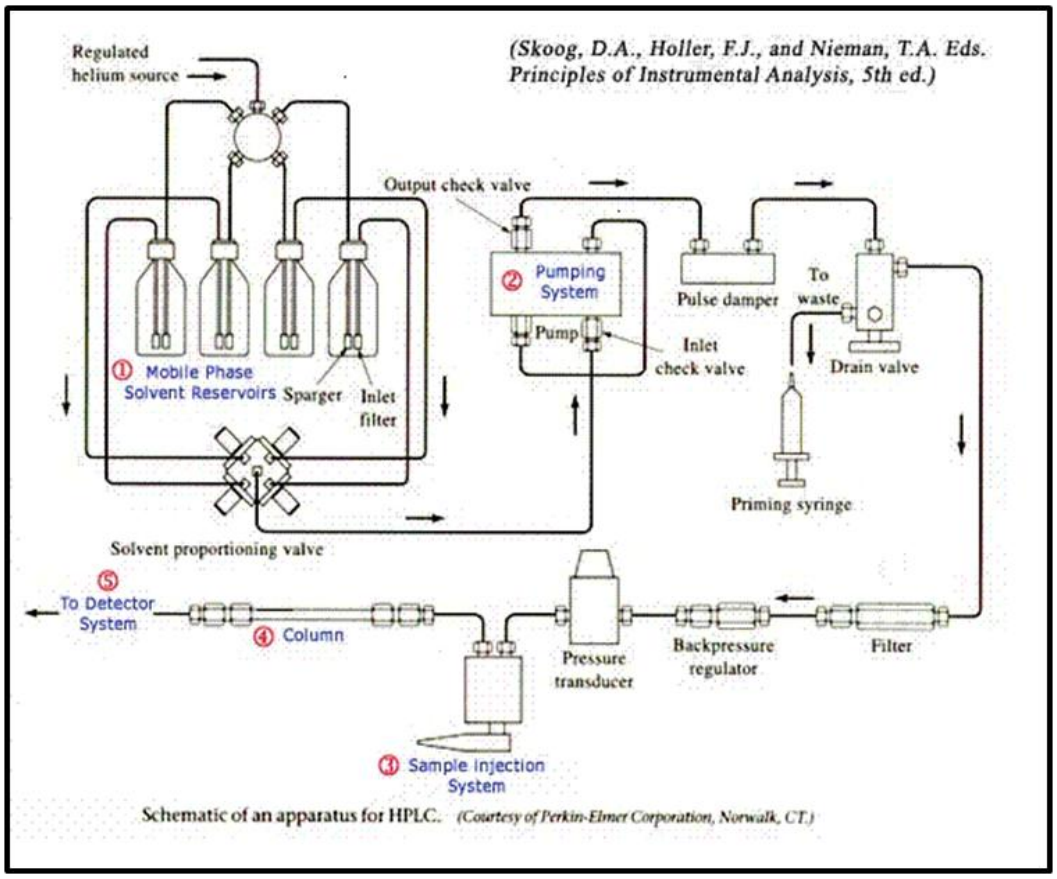
* **Normal phase mode**

In normal phase mode, the stationary phase (silica gel) is polar in nature and themobile phase is non-polar. In this technique, non-polar compounds travel faster and are eluted first. This is because of less affinity between solute and stationary phase.

* **Reverse phase mode**

In reverse phase technique, a non polar stationary phase is used. The mobile phase is polar in nature. Hence polar components get eluted first and non polar compounds are retained for a longer time. Since most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and eluted faster, which is advantageous.

1.4.3 INSTRUMENTATIONOF HPLC



Schematic of an apparatus for HPLC. *(Courtesy of Perkin-Elmer Corporation, Norwalk, CT.)*

* **Mobile phases / Solvent systems**

Each reservoir contains 200 to 1000 mL solvent (mobile phase) Mobile phase is normally degassed by any of the following techniques such as vacuum pumping system, vacuum filtration, distillation, heating, stirring, sonication, or sparging (gases swept away by inert, low solubility gas like He) which prevents band spreading and detector interference.

* **Pumping Systems**

High pressure pumps are required to force the solvents through packed stationary phase beds. An ideal pump should generate pressures up to 6000 psi (400 bar) with a pulse-free output giving a flow rate from 10 mL/min to less that 1µL/min. These types of pumps are available. They are Reciprocating pumps, Displacement pumps and Pneumatic pumps.

* **Injectors**

Injectors should provide the possibility of injecting the liquid sample within the range of 0.1 to 100 mL of volume with high reproducibility and under high pressure (up to the 4000 psi). They should also produce minimum band broadening and minimize possible flow disturbances. In liquid chromatography, the liquid samples may be injected directly and solid samples need only be dissolved in an appropriate solvent.

* **Columns**

Typical LC columns are made up of Stainless steel or glass tubing having a length varying form 10-30 cm fitted with extremely small diameter (3,5 or 10 µm) particles. The internal diameter of the column is usually 4 or 4.6 mm. However if pure substances are to be collected (preparative scale), larger diameter columns may be needed.

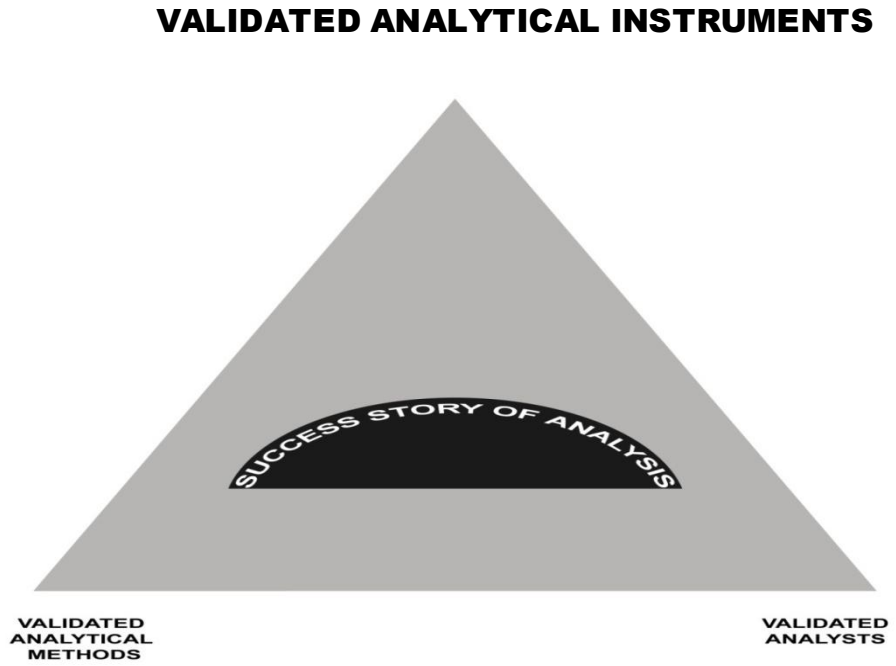
* **Detector Systems**

Detectors are used to visualize separated compounds and translate the concentration changes into signals.

The different types of detector used in HPLC methods based are ultraviolet (UV), fluorescence, refractive index, mass Spectrophotometric and electrochemical. In most cases, method development in HPLC is carried out with UV detection using a variable wavelength Spectrophotometric detector or a photo diode array detector (PDA).

1.5 VALIDATION

Validation of an analytical method is the process by which it is estimated, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.



"One of the most frustrating aspects for an analyst is working with an ill-defined, poorly designed and invalidated analytical method

1.5.1 REASONS FOR VALIDATION

* Enables scientists to communicate scientifically and effectively on technical matters.
* Setting standards of evaluation procedures for checking complaints and taking remedial measures
* Retrospective validation is useful for trend comparison of results compliance to cGMP/GLP.
* Closer interaction with pharmacopoeia harmonization particularly in respect of impurities determination and their limits.
* For taking appropriate action in case of non – compliance.
* To provide high degree of confidence that the same level of quality is consistently built into each unit of finished product from batch to batch.
* Economic: The consistency and reliability of validated analytical procedure is to produce a quality product with all the quality attributes, thus providing indirect cost saving from reduced testing or re testing and elimination of product rejection.
* As quality control process is not static, some form of validation / Verification should continue till the validated process is in use.

1.5.2 Summary of validation procedure

Type of validation	Test for
Specificity	Interference
Accuracy	Recovery ; linearity
Sensitivity	Limit of detection ; Limit of quantification
Precision	Repeatability ; Reproducibility ; Ruggedness
Personnel	Qualifications ; Experience ; responsibility ; proficiency
Equipment	Specifications, vendor, calibration, maintenance
Service	Sanitation, water, Waste disposal

1.6 STATISTICAL VALIDATION

1.6.1 Parameters used for Assay Validation

* **Specificity**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedures.

* **Accuracy**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

* **Precision**

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

* **Repeatability**

Express the precision under same operating conditions over a short interval of time. Repeatability is also termed as intra-assay precision.

* **Linearity**

Linearity of an analytical procedure is its ability (with in a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

* **Range**

Range of an analytical procedure is the interval between the upper and lower concentration (amount) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

* **Detection limit**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Based on the standard deviation of the response and the slope, the detection limit (DL) may be expressed as

$$DL = \frac{3.3\,\sigma}{S}$$

Where ,

σ = standard deviation of the response

S= slope of the calibration curve (of the analyte)

* **Quantification limit**

The quantification limit of an analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision, accuracy and reliability by the proposed method.

Based on the standard deviation of the response and the slope, quantitation limit may be expressed as

$$QL = \frac{10\,\sigma}{S}$$

Where ,

σ = standard deviation of the response

S = slope of the calibration curve (of the analyte)

* **System suitability testing**

System suitability is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

The system suitability testing parameters established for the liquid chromatographic procedure are:

* **Retention time (R_t)**

It is the time required by a sample component to migrate from column inlet to the column end.

* **Capacity factor (K')**

Chromatographic parameter which specifies the extent / degree of the retention time delay of a substance to be separated, called capacity factor

$$K' = \frac{t_1 - t_0}{t_0}$$

* **Peak asymmetry factor (A_s)**

Peak asymmetry factor, as can be used as a certerion of column performance.The peak half width because of a peak at 10% after peak height divided by the corresponding front half width a gives the asymmetry factor.

* **Tailing factor (T)**

The tailing factor T, a measure of peak symmetry, is unity for perfectly symmetrical peaks and its value increases as tailing becomes more pronounced.

In some cases, values less than unity may be observed. As peak asymmetry increases, integration, and hence precision becomes less reliable.

$$T = \frac{W_{0.05}}{2f}$$

Where,

$W_{0.05}$ = width of peak at 5% height

f = Distance from the peak maximum to the leading edge of the peak, the distance being measured at a point 5% of the peak height from the baseline.

Limit: ≤ 2 is preferable.

* **Number of theoretical plates (N)**

$$n = 16 \left[\frac{t}{w} \right]^2$$

The assessment of performance of efficient of a column is in terms of the number of theoretical plates.

* **Resolution**

Measure of quality of separation of adjacent bands, obviously overlapping bands have small R_S values, it is calculated from width and retention time of two peaks (or) separation of two peaks.

$$R_S = \left[\frac{2(t_2-t_1)}{W_2+W_1} \right]$$

* **Statistical parameters**

Statistics consist of a set of methods and rules for organizing and interpreting observations.

The precision or reproducibility of the analytical method was determined by repeating the analysis six times and the following statistical parameters were calculated.

The Formulas are

$$S = \sqrt{\frac{\sum_{i=1}^{i=n}(x_i - \bar{x})}{N-1}}$$

$$\text{R.S.D (\%)} = \frac{\text{S.D}}{\bar{x}} \text{ X } 100$$

$$\text{S.E} = \frac{\text{S.D}}{\sqrt{n}}$$

Where Σ = Sum of observations

$\bar{x}$ = Mean or arithmetic average ($\Sigma x / n$)

x = Individual observed value

$x - \bar{x}$ = Deviation of a value from the mean

n = Number of observations

Applications

To test the significance of the mean of a random sample

Testing difference between means of two samples(Independent samples)

Testing difference between means of two samples(Dependent samples)

Testing the significance of an observed correlation coefficient.

This statistics is versatile and can be applied in this present work to evaluate the validity of the proposed method.

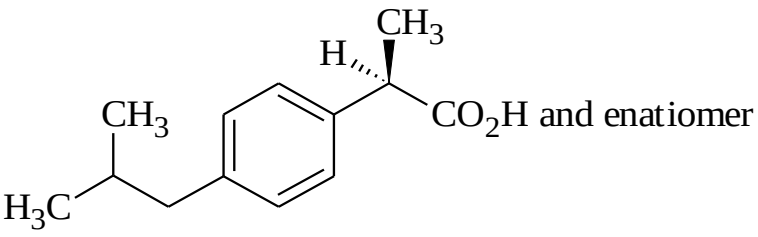
LITERATURE REVIEW

2.LITERATURE REVIEW

2.1 DRUG PROFILE

2.1.1 IBUPROFEN

Molecular Structure



Chemical Name

(2RS)-2-[4-(2-Methylpropyl)phenyl]propanoic acid.

Molecular Foumula

$C_{13}H_{18}O_2$

Molecular Weight

206.3g/mol

Category

Cyclo-oxygenase inhibitor; analgesic; anti-inflammatory.

Description

White or almost white; crystalline powder or colourless crystals.

Solubility

Practically insoluble in water, freely soluble in acetone, in methanol and in methylene chloride.

Identification

i) Melting Point

Standard Value	Observed Average Value*
75-78^{0}C	76^{0}C

*Average of six observations

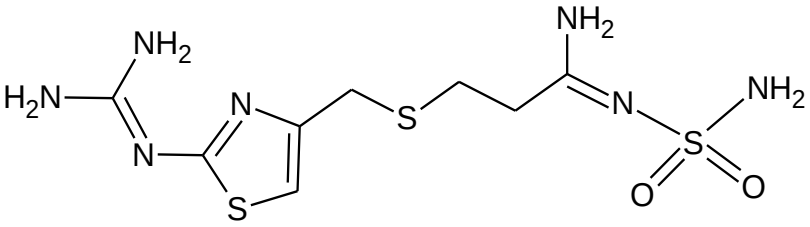
ii) IR Spectrum.

Storage

Store in a tightly closed container in a dry place.

2.1.2 FAMOTIDINE

Molecular Structure



Chemical Name

3-[[[2-[(Diaminomethylene) amino thiazol-4-yl] methyl] sulphanylN'-sulphamoylpropanimidamide.

Molecular Foumula

$C_8H_{15}N_7O_2S_3$

Molecular Weight

337.5g/mol

Category

Histamine H_2 receptor antagonist; treatment of peptic ulceration.

Description

White or yellowish-white, crystalline powder or crystals.

Solubility

Very slightly soluble in water, freely soluble in glacial acetic acid, slightly soluble in methanol.

Identification

i) Melting Point

Standard Value	Observed Average Value*
163-1640C	163^0C

*Average of six observations

ii) IR Spectrum.

Storage

Protected from light

2.2.REPORTED METHODS

2.2.1 REPORTED METHODS FOR IBUPROFEN

1. Hackett L.P and Duski L.J. et al., **(1978)**were reported **Gas-liquid chromatographic determination of Ibuprofen in human plasma.**
2. Viddesh R, Bavi M,Sundaresan and Dhorda V.J**.** et al., **(1997)**were reported **A simultaneous packed column supercritical fluid chromatographic method for Ibuprofen, chlorzoxanzone and acetaminophen in bulk and dosage forms.**
3. Frinmat R, Boucher M and Bussac E**,** et al., **(1999)**were reported**Bio inversion of Ibuprofen enatiomers after administration in dogs.**
4. Knihinicki D and Richard. et al., **(2004)**were reported **Steroselective disposition of Ibuprofen and flurbiproten in rats.**
5. Franciszek,Glowka K and Marta. et al., **(2005)**were reported **High performance capillary electrophoresis method for determination of Ibuprofen enantiomers in human serum and urine.**
6. Chitlange S.S, Sakarkar A.M and Wadodkar S.A.et al., **(2008)** were reported **HPLC method for simultaneous estimation of Ibuprofen and pseudoephedrine HCL**. This chromatographic method was achieved on C_{18} column with a mixture of methanol:Acetonitrile: water (60:15:25 v/v/v) using a UV-PDA detector at 260 nm.
7. Prasanna Reddy, and Reddy M.S. et al., **(2009)**were reported **RP-HPLC method for simultaneous estimation of paracetamol and Ibuprofen in tablets**. This chromatographic method was achieved on C_{18} column with a mixture of acetonitrile and phosphate buffer pH 4.0 (70:30v/v) and the detection is carried out using a UV-PDA detector at 248 nm.

8. Thomas A.B , Oumbre N.G, and Deshpande A.D.et al., **(2009)**were reported **Simultaneous determination of tramadol and Ibuprofen in pharmaceutical preparation by 1st order derivative method and LC method.**
9. RiddhiGondalia and PankajSavaliya. et al., **(2010)**were reported **Development and validation of spectrophotometric methods for simultaneous estimation of Ibuprofen and paracetamol in soft gelatin capsule by simultaneous equation method.**
10. NarasimhaSwamyLakka and NishantGoswami,et al., **(2011)**were reported **development and validation of RP-HPLC for simultaneous determination of Ibuprofen and Paracetamol in solid dosage forms**. This chromatographic method was achieved on $_{C18}$ column with a mixture of acetonitrile and phosphate buffer pH 6.0 (80:20 v/v) and the detection is carried out using a UV-PDA detector at 260 nm.

2.2.2 REPORTED METHODS FOR FAMOTIDINE

1. Clakir B, Tosun A.U. and Sahin M.F. et al., **(1997)**were reported **Quantitative HPLC analysis of Famotidine in Pharmaceutical dosage forms.** This chromatographic method was achieved on $_{C18}$ column with a mixture of acetonitrile and phosphate buffer pH 6.0 (80:20 v/v) and the detection is carried out using a UV-PDA detector at 248 nm.
2. Novakovic J. et al., **(1999)**were reported **HPLC for the determination of ranitidine hcl and famotidine in pharmaceuticals.** This chromatographic method was achieved on $_{C18}$ column with a mixture of acetonitrile and phosphate buffer pH 6.5 (85:15 v/v) and the detection is carried out using a UV-PDA detector at 260 nm.

3. Volkanzaimogia and ZelinagarDegim, et al., **(2001)**were reported **pH – metric logk calculation of Famotidine, naproxen, nizatidine, ranitidine and salicylic acid.**
4. Zelihaguldegim and Illbeyi, et al., **(2002)**were reported **nonisothermal stability test of Famotidine and gizatidine.**
5. NafisurRahman and Mohammad Kashif. et al., **(2003)**were reported **Spectrophotometric determination of Famotidine in drug formulation.**
6. Sahu R, Batachariya S and Deepti Jain. et al., **(2006)**were reported **Simultaneous spectro photometric estimation of Famotidine and domperidone in combined dosage form.**
7. Arayne, Saeed M and Farhan Ahmed. et al., **(2010)**were reported **Simultaneous determinations of metformin, cimiatidine, Famotidine and ranitidine in human serum and dosage formulation using HPLC with UV detection.**
8. Ok Ram Zenita Devi and KanakapuraBasavaiah. et al., **(2011)**were reported **Simple and sensitive UV spectrophotometric methods for determination of Famotidine in tablet formulation.**
9. Zenita O and Basavaiah K. et al., **(2011)**were reported **Utility of N.Bromosuccinimide for the titimetric and spectrophotometric determinations of Famotidine in pharmaceutical formulation.**
10. Dipali D. Tajane, Gadnave V and Chodhari P**.** et al., **(2011)**were reported **Spectrophotometric simultaneous determination of Famotidine and domperidone in combined tablet dosage form by ratio derivative and area under the curve method.**

AIM AND PLAN OF WORK

3.Aim and Plan of Work

The combination dosage form selected for the present study contains Ibuprofen and Famotidine in solid oral dosage forms, recently this combination has been approved by CDSCO (Central Drug Standard Organisation)

Literature survey reveals that UV, HPLC and GCmethods are reported for the determination of ibuprofen and famotidine individually and combination with some other drugs. There are no reported UV and RP-HPLC methods for simultaneous estimation of both drugs in combined dosage form.

Hence an attempt has been made to develop simple sensitive, rapid, accurate and precise for the simultaneous estimation of ibuprofen and famotidine in bulk and in tablet dosage form.

For UV method,

1.Find the drugs solubility in various solvents

2.To determine maximum absorbance and overlaid the spectrum

3. Selection of wavelength from the overlaid spectrum

4.Determining the standard absorbance for all selected wavelength for each drugs

5.Development of simple, precise, accurate and sensitive

Area under the curveand simultaneous equation method

Derivative method, in the specified range

6.Validation of development as per ICH guidelines

For RP-HPLC method,

1. A suitable mobile phase were selected for two drugs with proper resolution and short duration time.

2. Development of chromatogram with various concentration for eachdrug.

3. Development of chromatogram in formulation.

4. Validation of the developed method.

MATERIALS

&

METHODS

4. MATERIALS AND METHODS

4.1 MATERIALS

4.1.1 Drug Sample

Ibuprofen and Famotidine was obtained as a gift sample from Medo Pharm Pvt. Ltd., Guduvancherry.

4.1.2 Formulation Used

Tablets were prepared in Medo Pharm Pvt. Ltd., Guduvancherry. It contains ibuprofen 800 mg and famotidine 26.6 mg

4.1.3 Chemicals and Solvents used

All the following chemicals used were of analytical and HPLC grade.

1. Methanol
2. Distilled water
3. Acetonitrile (HPLC grade)
4. Water for HPLC (Millipore and MilliQ)
5. Potassium Di-hydrogen phosphate
6. Ortho phosphoric acid

Chemicals and solvents were procured from Qualigens India Pvt. Ltd, Merck and LobaChemie India Ltd.

4.1.4 Instruments Used

Instruments employed for the study were,

SHIMADZU AUX - 220 Digital Balance

SHIMADZU – 1700 Double Beam - UV – Visible spectrophotometer with pair of 10 mm matched quartz cells.

SHIMADZU HPLC (UFLC)

LC-20AD

PDA-Detector

Software – Shimadzu LC solutions

Lab India – pH meter

Sartorius – Digital Balance

Melting point apparatus

4.1.5 Instruments Specifications

A) Shimadzu AUX – 220 digital balance: (Shimadzu instruction manual)

Specifications	
Weighing capacity	200 gms
Minimum display	0.1 mg
Standard deviation	≤ 0.1 mg
Operation temperature range	5 to 40° C

B) Shimadzu UV – Visible spectrophotometer: (Shimadzu instruction manual)

Model: Shimadzu, UV-1700; Cuvetts: 1 Cm quartz cells.

Specifications	
Light source	20 W halogen lamp, Deuterium lamp. Light source position automatic adjustment mechanism.
Monochromator	Aberration-correcting concave holographic grating
Detector	Silicon Photodiode
Stray Light	0.04% or less (220 nm: NaI 10 g/l) 0.04% or less (340 nm: $NaNo_2$ 50 g/l)
Measurement wavelength range	190~1100 nm
Spectral Band Width	1 nm or less (190 to 900 nm)
Wavelength Accuracy	± 0.5 nm automatic wavelength calibration mechanism
Recording range	Absorbance : -3.99~3.99 Abs Transmittance : -399~399%
Photometric Accuracy	± 0.004 Abs (at 1.0 Abs), ±0.002 Abs (at 0.5 Abs)
Operating Temperature/Humidity	Temperature range : 15 to 35°C Humidity range : 35 to 80% (15 to below 30° C) 35 to 70% (30 to 35° C)

C) Shimadzu High Performance Liquid Chromatography: (Shimadzu instruction manual)

Detector Specifications	
Light source	Deuterium Arc lamp
Measurement wavelength range	190 to 700 nm
Spectral Band Width	5 nm
Wavelength Accuracy	± 1 nm
Cell path length	10 nm
Cell volume	20 µl
Operating temperature range	4 to 35° C (39 to 104° F)
Recording range	0.0001 to 4.000 AUFS
Operating temperature/Humidity	4 to 35° C / 75 %
Pump Specifications	
Pump type	Double reciprocating plunger pump
Pumping method	Constant flow delivery and constant pressure delivery
Suction filter	45 µm
Line filter	5 µm mesh
Operating temperature	4 to 35° C (39 to 104° F)

4.2 METHODS

Methods employed for the determination of ibuprofen and famotidine

In the present work an attempt was made to develop and validate a simple, precise and accurate method for the estimation of ibuprofen and famotidine in pure and in combined tablet dosage form by UV- spectrophotometry and RP-HPLC method.

4.2.1 UV-Spectrophotometry

A. Area under the curveand simultaneous equation method

B. Derivative spectrophotometric method

4.2.2 RP-HPLC

*** SPECTROPHOTOMETRY METHODS**

SELECTION OF SOLVENT

The solubility and stability for both ibuprofen and famotidine were evaluated. The absorbance of both drugs were higher and exhibited distinct λmax in methanol.

PREPARATION OF STANDARD STOCK SOLUTION

The stock solution of 1mg/mL of each of ibuprofen and famotidine were prepared in methanol. Further dilutions were prepared in methanol.

SELECTION OF WAVELENGTH

For the selection of wavelength for the estimation of ibuprofen and famotidine, a suitable standard solution contain 10 mcg/mL of ibuprofen and famotidine were prepared individually and scanned in the entire range from 200-400 nm, an overlaid spectra was made. From the overlaid spectra of ibuprofen and famotidine, area were measured at

wavelengths 227.5 – 220 nm and 294 – 282 nm for the determination of ibuprofen and famotidine respectively.

For derivative spectroscopic method, the zero order spectrum was derivatised to first order spectrum in that 209.5 nm was selected for the estimation of ibuprofen, which is zero crossing for famotidine and 248 nm was selected for the estimation of famotidine which is zero crossing for ibuprofen.

LINEARITY AND CALIBRATION FOR AREA UNDER THE CURVE AND DERIVATIVE METHOD

Linearity of the method was studied by plotting calibration curves. From each of the stock solutions suitable aliquots were diluted to get concentrations in the range of 15 – 75 mcg/mL for ibuprofen and 0.5-2.5 mcg/mL for famotidine. Both sets of solutions were scanned in the range of 200 nm – 400 nm. Area were measured at 227.5-220 nm and at 294-282 nm for ibuprofen and famotidine respectively for area under the curve method. Then in the first derivative mode, $\Delta A/\Delta\lambda$ of the same solutions were measured at 209.5 nm for ibuprofen and 248 nm forfamotidine for derivative spectroscopy method. Calibration curves were obtained by plotting absorbance against concentration.

QUANTIFICATION IN FORMULATION

Twenty tablets were weighed accurately, average weight was found and crushed to fine powder. The powder equivalent to 300 mg of ibuprofen and 10 mg of famotidine was transferred to 25 mL volumetric flask. The powder was dissolved in 10 mL of methanol by intermittent shaking and volume was made up to 25 mL with the same solvent. The solution was then filtered through a whatmann filter paper no.41. The

resultant solution was diluted to 30 mcg/mL of ibuprofen and 1 mcg/mL of famotidine by methanol.

The concentration of both ibuprofen and famotidine were determined by measuring the area of samples at 227.5-220 nm and 294-282 nm for area under the curve method and $\Delta A/\Delta\lambda$ at 209.5 nm and 248 nm (in first derivative mode) for derivative spectrophotometric method and the amount were found by the area in the simultaneous equation for the area under the curve method and respective calibration graph for derivative spectroscopic method.

VALIDATION

LINEARITY

A calibration curve was plotted between concentration and absorbance. Ibuprofen was linear with the concentration range of 15-75 mcg/mL and famotidine showed the linearity in the range of 0.5-2.5 mcg/mL.

ACCURACY (Recovery Studies)

To ascertain the accuracy of proposed methods, recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). Percent recovery for ibuprofen and famotidine was in the range of (99.29%-101.13%) by area under the curve method and was in the range of (99.00 % - 101.87%) by derivative spectroscopic method. The accuracy and reproducibility is evident from the data as results are close to 100% and low standard deviation value. The amount of each drug recovered was calculated. This procedure was repeated for three times for each concentration. The % RSD was calculated.

PRECISION

Precision of the method was demonstrated by repeatability studies. Repeatability studies were done by consequently analyzing the sample solution for six times. The amount of each drug present in the tablet formulation was calculated. The % RSD was calculated. Intra day and inter day precision were established by repeating the determination on the same day and on three successive days respectively. The amount of drugs was determined and % RSD also calculated.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Preparation of calibration curve from the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification was calculated by using the average value of slope and standard deviation of response.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD

Chromatographic method depends up on the nature of the sample, molecular weight and solubility. The drug selected for the present study was polar compound, hence it can be separated either by normal phase or reverse phase chromatography. Reverse phase chromatographic technique was selected for initial separations with the knowledge of properties of compound, C_8 column was chosen as stationary phase and various mixtures of phosphate buffer (pH-6.5) and acetonitrile (85:15) were considered as mobile phase.

SELECTION OF MOBILE PHASE AND WAVELENGTH

Different mixtures of mobile phase with different ratios were selectedand their chromatograms were recorded. From this phosphate buffer (pH-6.5)Acetonitrile (85:15) was selected as mobile phase, since these two drugs were eluted with sharp peak and with better resolution. Hence this mobile phase was used to optimize the chromatographic conditions.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

The following parameters were used for RP-HPLC analysis of Ibuprofen and Famotidine.

Mode of operation	- Isocratic
Stationary phase	- C_8 column (150mm × 4.6 mm, 5mc)
Mobile phase	- Phosphate buffer (pH-6.5) : Acetonitrile
Ratio	- 85:15
Detection wavelength	- 265 nm
Flow rate	- 1 ml / min
Temperature	- Ambient
Sample volume	- 10 µg/ml
Operating pressure	- 150 kgf

PREPARATION OF THE STANDARD STOCK SOLUTION

An accurately weighed ibuprofen (300 mg) and famotidine (10 mg) were transferred to 100 mL volumetric flask, dissolved in 50 mL methanol and diluted up to mark with methanol. The standard stock solution had concentrations of ibuprofen (3000 mcg/mL) and famotidine (100 mcg/mL), Accurately measured 2.5 mL of standard stock solution was transfer to 25 mL volumetric flask and diluted up to the mark with methanol The working standard solution had concentrations of ibuprofen (300 mcg/mL) and famotidine (10 mcg/mL)

LINEARITY AND CALIBRATION

Aliquots (1.0, 1.5, 2.0, 2.5, and 3.0 mL) of mixed working standard solutions of ibuprofen and famotidine each were transferred in a series of 10 mL volumetric flasks, and the volume was made up to the mark with methanol. An aliquot (10 mcL) of each solution was injected under the operating chromatographic condition as described above and responses where recorded. Calibration curves were constructed by plotting the peak areas versus the concentration, and the regression equations were calculated. Each response was average of three determinations.

QUANTIFICATION OF IBUPROFEN ANDFAMOTIDINE

For determination of the content of ibuprofen and famotidine in tablets; twenty tablets were weighed accurately and average weight was found and crushed to fine powder. The powder equivalent to 300 mg ibuprofen and 10 mg of famotidine was transferred to a volumetric flask and dissolved in 60 ml of methanol. The solution was sonicated for 30 min. The extracts were filtered through nylon filter paper and the residue was washed thoroughly with methanol. To ensure completion the solution was filtered again through 0.45 mc filter paper, the extracts and washing were pooled and

transferred to a 100 mL volumetric flask and volume was made up to 100 mL with methanol. Accurately measured 1.0 mL of standard sample stock solution was transferred to10 mL volumetric flask, diluted up to the mark with methanol to get final working concentration of ibuprofen (300 mcg/mL) and famotidine(10 mcg/mL) An aliquot (10 mcL) of sample solution was injected under the operating chromatographic conditions as described above and responses were recorded. The analysis procedure was repeated six times with tablet formulation.

RECOVERY STUDIES

The accuracy of the method was determined by calculating the recoveries of ibuprofen and famotidine by the standard addition method. Known amounts of standard solutions of ibuprofen and famotidine were added at 80, 100, 120 % level to prequantified sample solution of ibuprofen and famotidine (300 mcg/mL) of ibuprofen and (10 mcg/mL) of famotidine. The amounts of ibuprofen and famotidine were estimated by applying obtained values to the respective regression equations.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Preparation of calibration curve for the serial dilution of standard was repeated for six times. The limit of detection and limit of quantification were calculated by using the average value of slope and standard deviation of response (Intercept).

SYSTEM SUITABILITY STUDIES

The system suitability studies were carried out as specified in B.P. the parameter like Column efficiency, Resolution, Tailing factor, Asymmetric factor, and Theoretical plate number were calculated.

RESULTSAND DISCUSSION

5. RESULTSANDDISCUSSION

ABOUT UV SPECTROSCOPIC METHODS

In order to quench the thirst for the analysis of a new drug combination, the drug ibuprofen and famotidine were taken for our studies. To ensure the percentage purity in combined dosage form, the UV-Visible spectroscopic and RP-HPLC methods were developed. The methods were very simple, economic and applicable for routine analysis.

5.1 UV-SPECTROSCOPIC STUDIES

The solubility of ibuprofen and famotidine were determined in a variety of solvents using ScHefter and Higuchi method. 10 mg samples were taken in test tube and checked their solubility with variety of solvents as per IP and the profiles were shown in Table-1.

From the solubility studies, methanol was chosen as solvent for UV- Visible spectroscopic studies in bulk and in formulation. Based upon its easy availability, cost factor and the stability conditions methanol was selected as solvent.

Two simple, sensitive and precise UV methods namely, Area under the curveand simultaneous equation method, and Derivative spectroscopic methods were selected for the determination of ibuprofen and famotidine in pharmaceutical formulations.

The drugs were dissolved in methanol to produce 10 mcg/mL. Scanned in the range of 200-400 nm and it shows constant wavelength at 222.0 nm for ibuprofen and 290.0 nm for famotidine and overlain spectra was made. This is shown in Fig. 3, 4 and 5 respectively. Stability of absorbance at their wavelength was also checked.

The linearity of ibuprofen and famotidine was constructed in the range of 15-75 and 0.5-2.5mcg/mL and their calibration curves were shown in the Fig. 6 to 9respectively. The optical characteristics such as Beer's law limit (15-75 and 0.5-2.5mcg/mL), molar extinction co-efficient, sandell's sensitivity, correlation co-efficient, slope and intercept were calculated and are shown in Table-2 to 4.

The formulation was selected for analysis. The amount present were determined by calculating the average of six replicate analysis and its percentage purity was found to be in the range of 98-102 % by the two methods. It is shown in Table- 5 and 6 respectively.

To evaluate the accuracy of the method, recovery studies were carried out, known amount of pure drug was added to the pre-analyzed solution containing formulation and the mixture was re-analyzed by the proposed methods, and their recoveries were calculated. The percentage recovery of ibuprofen and famotidine in the formulation were found to be in the range of 98-102%. These values are shown in Table-10 and 11.

Precision of the method was studied by making repeated analysis of the same sample and it was carried out three times in a day and for three days. The % RSD and standard deviation for inter-day and intra day analysis was found to be less than 2 indicates the method is precise, which are shown in Table 7 and 8.

The developed method was validated for Ruggedness. In the present work it was confirmed by different analysts. The % RSD value by analyst 1 and analyst 2 were found to be 1.3954 and 0.7188 for famotidine and 0.7241 and 0.7637 for ibuprofen respectively. The low % RSD values indicate that the developed method was more rugged. The results are shown in Table - 9.

The limit of detection and the limit of quantification were determined from the linearity studies which was done 6 times and calculated by using slope and standard deviation of response (Intercept).

5.2 RP-HPLC METHOD

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for ibuprofen and famotidine were obtained with a mobile phase comprising of phosphate buffer : acetonitrile (85:15 V/V) at a flow rate of 1.0 mL min^{-1} to get better reproducibility and repeatability with resolution (0.000 and 40.210), tailing factor (1.082 and 0.795), asymmetric factor (0.956 and 0.582), theoretical plate per unit(56053 and 1950) all the values are within the limit as per ICH guidelines for ibuprofen and famotidine respectively. Quantification was achieved with PDA detector at 265 nm based on peak area. The retention time for famotidine and ibuprofen were found to be 2.4 and 10.2 min, respectively (Fig.1). The results of system suitability parameters are given in (Table 13).

Linear correlation was obtained between peak area versus concentrations of ibuprofen and famotidine in the concentration ranges of 300-900 and 10-30 (mcg/mL) respectively. The correlation coefficient were 0.9998 for ibuprofen and 0.9991 for famotidine was obtained (Tabel12).The linearity chromatogram for ibuprofen and famotidine are shown in the fig. 15 to 19.The % RSD values for ibuprofen and famotidine were found to be <2% which indicates that the proposed method is repeatable, reveal that the proposed method is precise.

The accuracy of developed method was found to be 98.75-100.17 for ibuprofen and 99.34-99.80 for famotidine are shown in the (Table 15) which indicates accuracy of the proposed method.

The precision of the method was confirmed by repeatability of formulation for six times and the chromatograms are shown inFig.23-28.The %RSD were found to be 0.9499 and 1.6388 for ibuprofen and famotidine respectively. This data shown inTable 14.

LOD values for ibuprofen and famotidine were found to be 0.0872 mcg/mLand 0.0366 mcg/mL respectively and LOQ values for ibuprofen and famotidine were found to be 0.0264 mcg/mL and 0.0999 mcg/mLrespectively.

These data show that the proposed method is sensitive for the determination of ibuprofen and famotidine . It was observed that there is no interference of the excipients with the principal peak. Hence, the method is specific for the estimation ibuprofen and famotidine. The results of stability indicated no significant change in the assay results of the same solution (% RSD is less than 2) the confirming the stability of the drug in the solvent used for the analysis.

The amount of ibuprofen and famotidine present in the sample solutions were determined by fitting the responses into the regression equations of the calibration curve for ibuprofen and famotidine, respectively and the results obtained were comparable with the corresponding labelled claim.

SUMMARY AND
CONCLUSION

6. SUMMARY AND CONCLUSION

Ibuprofen is chemically (RS) – 2 – (4-(2- methyl propyl) Phenyl) propanoic acid[1]. It is a nonsteroidal anti – inflammatory drug (NSAID) used for relief of symptoms' of arthritis, fever and analgesic (pain reliever) especially where there is an inflammatory component and dysmenorrhea.

Famotidine is chemically 3 – [[[2-(Diaminomethylen] amino] thiazol – 4 – yl] methyl]sulphanyl N – sulphamoyl propanimidamide[1]. It is a histamine H_2 – receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of peptic ulcer diseases and gastroesophageal reflux disease. Combination of ibuprofen and famotidine is anti inflammatory and reduce the risk of ulcer.

The proposed analytical methods are simple, economical, rapid, sensitive, reproducible and accurate for the simultaneous estimation of ibuprofen and famotidine. The methods adopted for studies were,

6.1 UV- SPECTROSCOPIC METHOD

The drug samples containing ibuprofen and famotidine in combined dosage forms were analyzed by UV-spectroscopic method using methanolas a solvent and the contents of drug determined in each formulations was found to be satisfactory.

UV-spectroscopic method for the estimation of ibuprofen and famotidine in combined dosage form by

Area under the curve and simultaneous equation method

Derivative spectroscopic method

Area under the curve and simultaneous equation method

The percentage label claim present in tablet formulation was found to be 100.01% and 100.14% for ibuprofen and famotidine respectively. The percentage recovery was found to be in the range of 99.79-100.83%.

Derivative spectroscopic method

The percentage label claim present in tablet formulation was found to be 100.13% and 100.47% for ibuprofen and famotidine respectively. The percentage recovery was found to be in the range of 99.00-101.87%.

6.2 RP-HPLC method.

RP-HPLC method has been developed for the estimation of both drugs in bulk and in formulation. The proposed method gives reliable assay results with short analysis time, using mobile phasephosphate buffer (pH-6.5): acetonitrile in the ratio of (85:15). The contents of drug present in the formulation were found to be satisfactory and system suitability parameters are in desired limit.

All the above methods do not suffer from any interference due to common excipients. It indicates that methods were accurate. Therefore the proposed methods could be successfully applied to estimate commercial pharmaceutical products containing ibuprofen and famotidine.

Thus the above studies findings would be helpful to the analytical chemists to apply the analytical methods for the routine analysis of the analyte in pharmaceutical dosage forms after their approval from FDA.

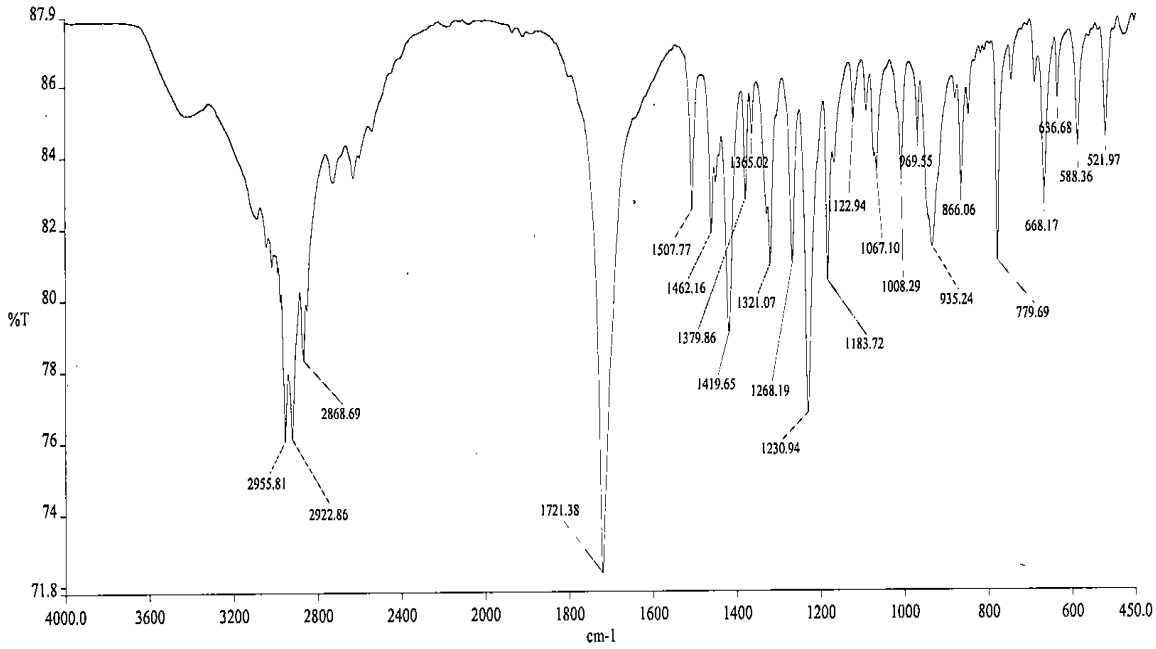
FIGURES

Fig – 1

IR SPECTRUM OF IBUPROFEN

Medopharm pvt ltd
instrument model: Spectrum 100

FTIR Spectrum
A.R.Number: Enclosure:



SAMPLE SPECTRUM

description: ibuprofen

spectrum name: PROJECT.007

spectrum pathname: PROJECT.007

date created: Monday, December 19, 2011 11:52 AM India Standard Time

The IR spectrum of the sample is _____ with the reference spectrum of _____

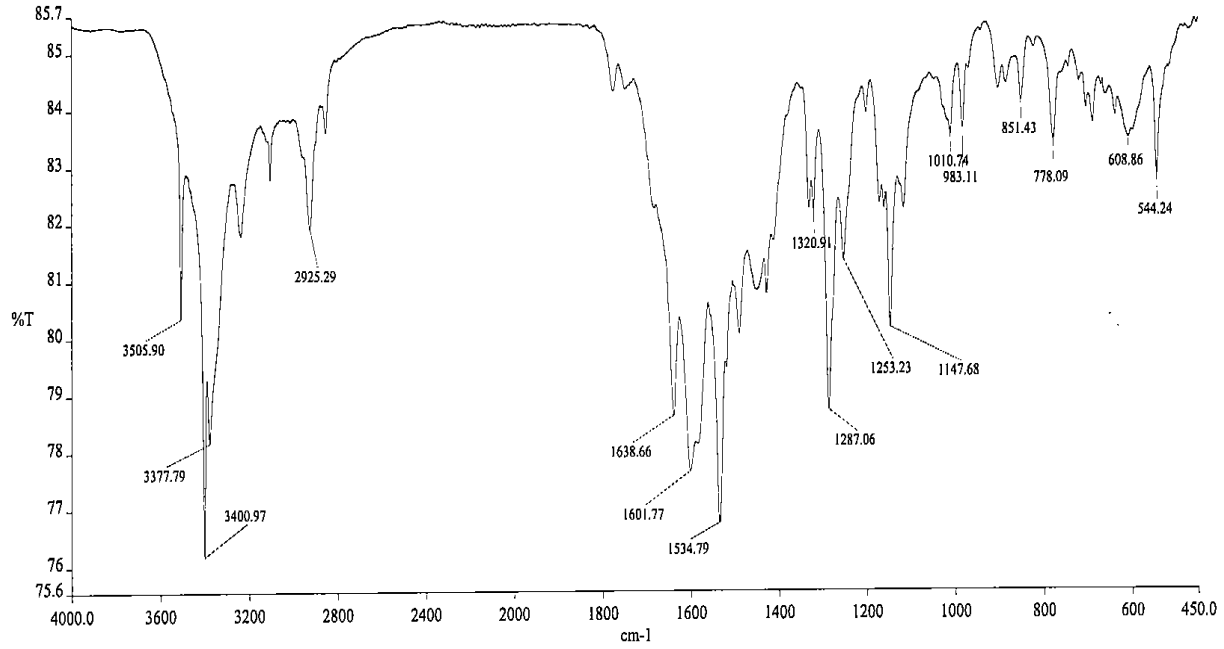
Analysed by: Checked by: Page 1 of 1

Fig – 2

IR SPECTRUM OF FAMOTIDINE

Medopharm pvt ltd
instrument model: Spectrum 100

FTIR Spectrum
A.R.Number: Enclosure:



SAMPLE SPECTRUM

description: Famotidine

spectrum name: PROJECT.003

spectrum pathname: PROJECT.003

date created: Monday, December 19, 2011 11:44 AM India Standard Time

The IR spectrum of the sample is _____ with the reference spectrum of _____

Analysed by: Checked by: Page 1 of 1

Fig – 3

UV SPECTRUM OF IBUPROFEN IN METHANOL AT 222.0 nm

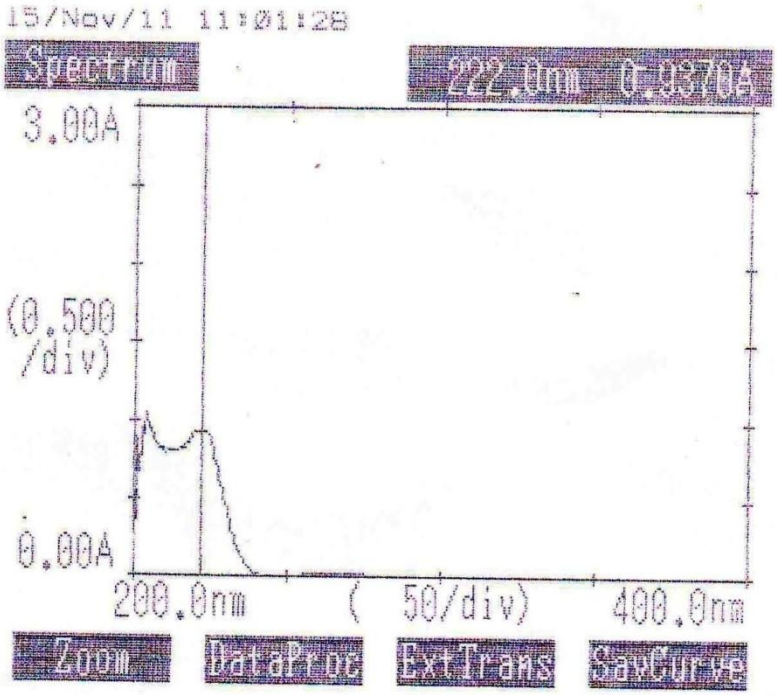


Fig - 4

UV SPECTRUM OF FAMOTIDINE IN METHANOL AT 290.0 nm

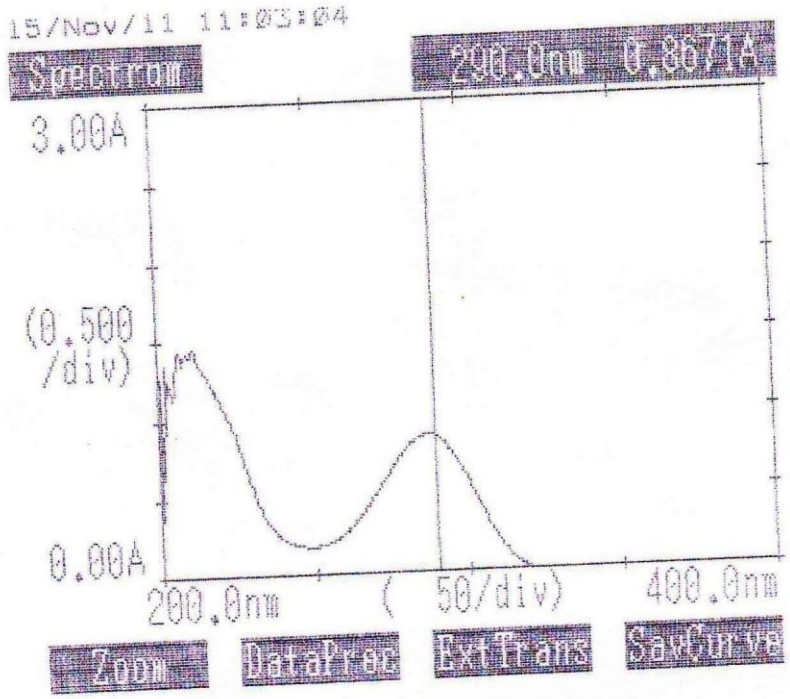


Fig – 5

OVERLAID ABSORPTION SPECTRA OF IBUPROFEN AND FAMOTIDINE IN METHANOL

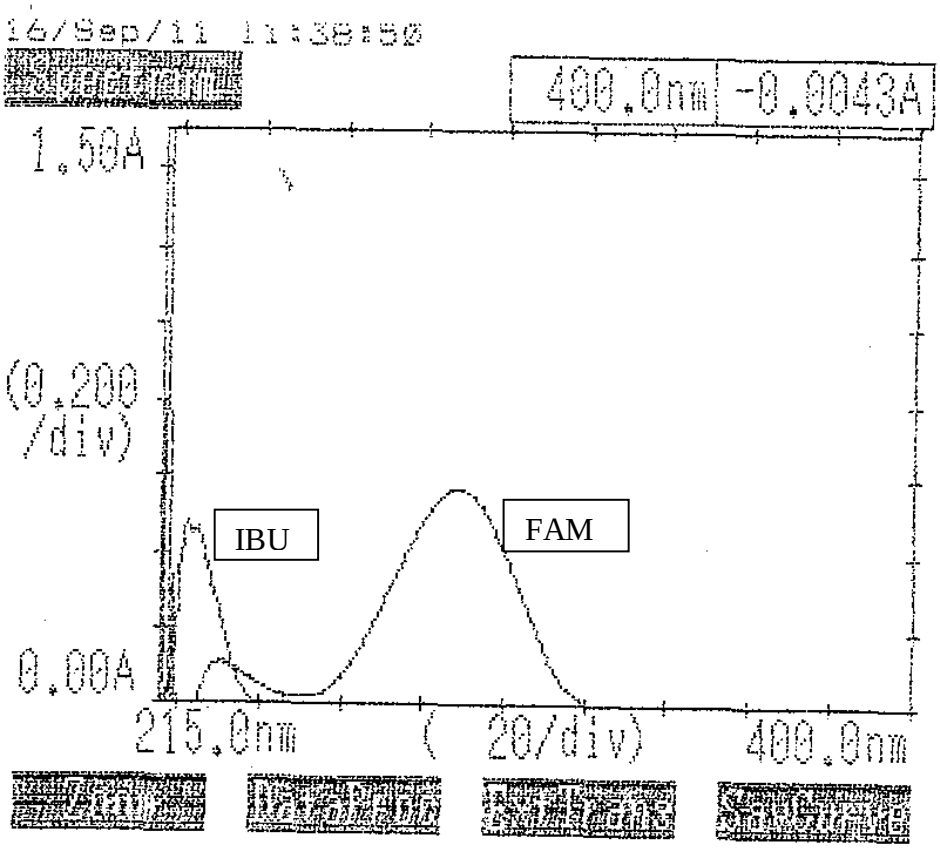


Fig – 6

CALIBRATION CURVE OF FAMOTIDINE IN METHANOL AT 220-227.5 nm

(AREA UNDER THE CURVE AND SIMULTANEOUS EQUATION METHOD)

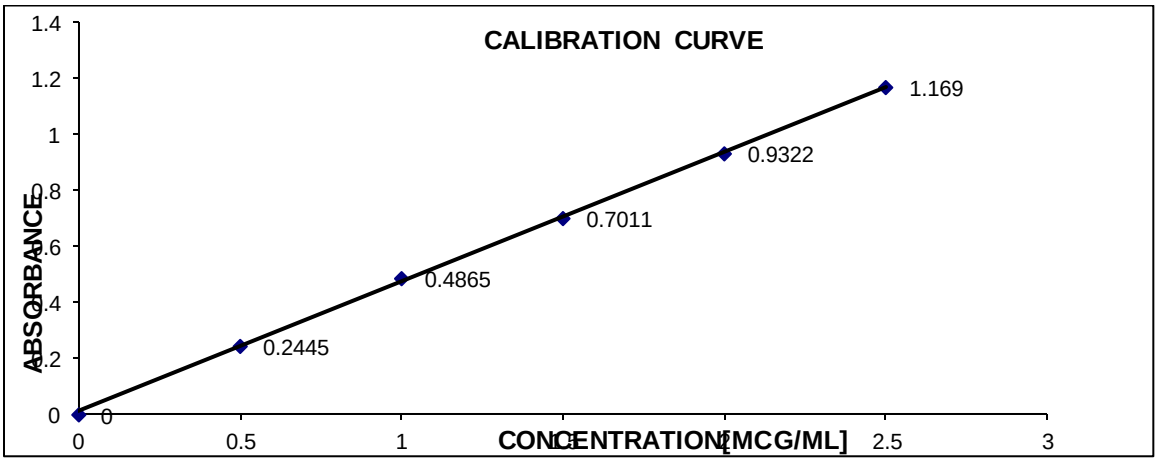


Fig – 7

CALIBRATION CURVE OF FAMOTIDINE IN METHANOL AT 282-294 nm

(AREA UNDER THE CURVE AND SIMULTANEOUS EQUATION METHOD)

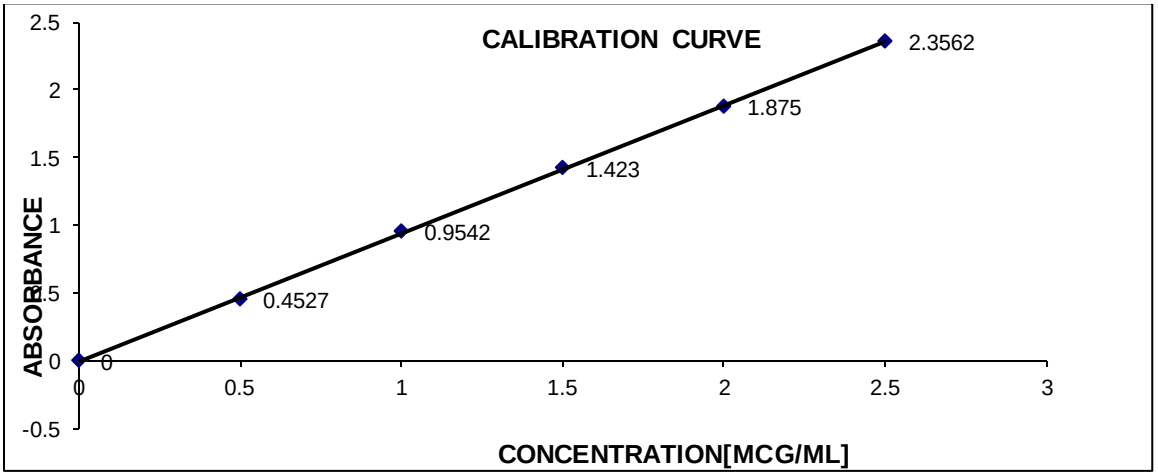


Fig - 8

CALIBRATION CURVE OF IBUPROFEN IN METHANOL AT 220-227.5 nm

(AREA UNDER THE CURVE AND SIMULTANEOUS EQUATION METHOD)

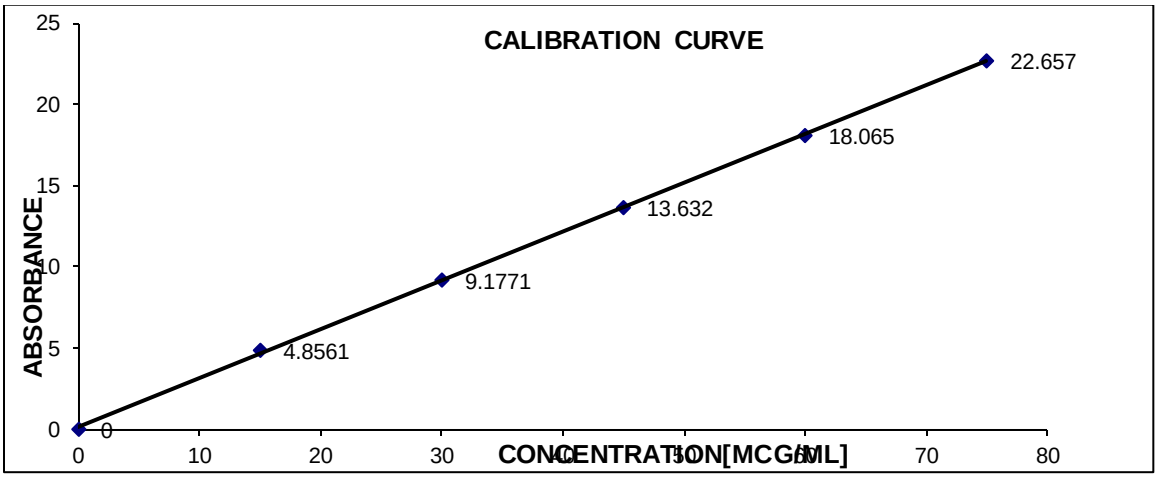


Fig - 9

CALIBRATION CURVE OF IBUPROFEN IN METHANOL AT 282-294 nm

(AREA UNDER THE CURVE AND SIMULTANEOUS EQUATION METHOD)

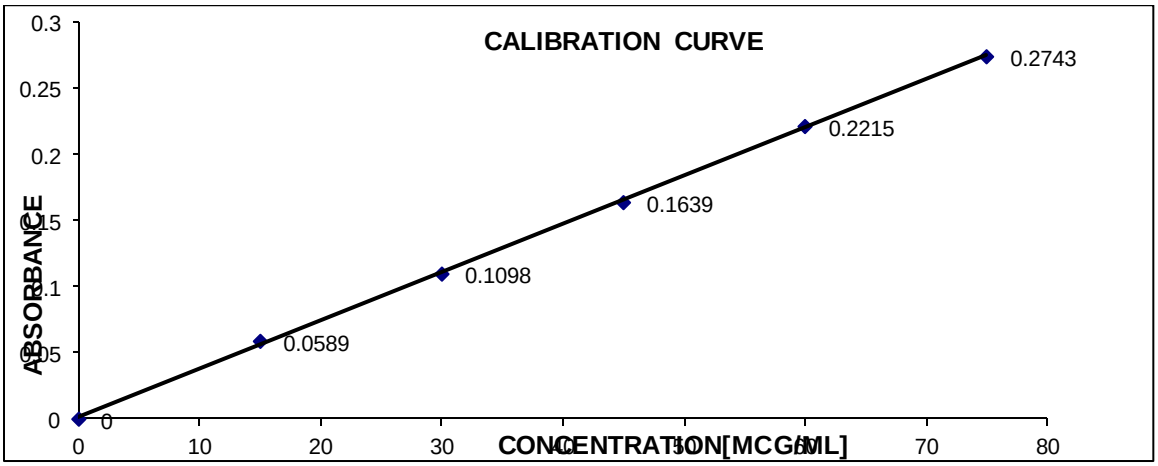


Fig – 10

FIRST ORDER DERIVATIVE UV-SPECTRUM OF FAMOTIDINE IN METHANOL

(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

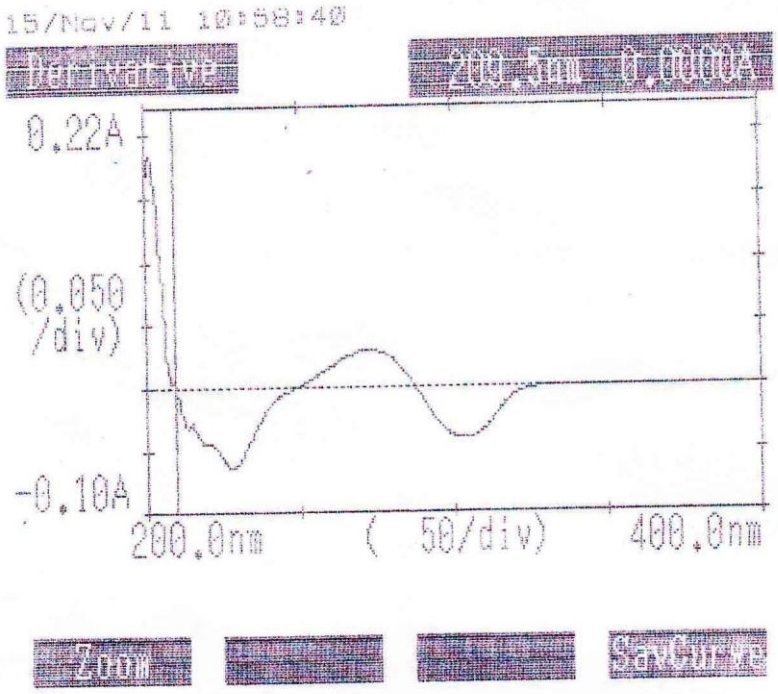


Fig –11

FIRST ORDER DERIVATIVE UV-SPECTRUM OF IBUPROFEN IN METHANOL

(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

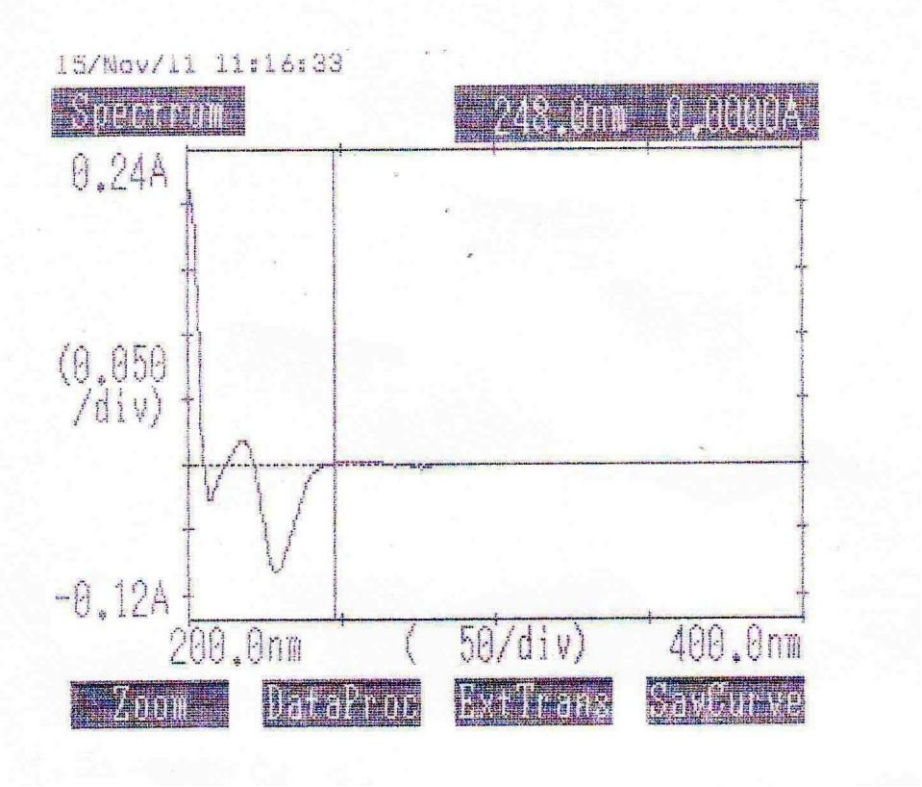


Fig – 12

OVERLAID FIRST ORDER DERIVATIVE UV-SPECTRUM OF IBUPROFEN AND FAMOTIDINE IN METHANOL

(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

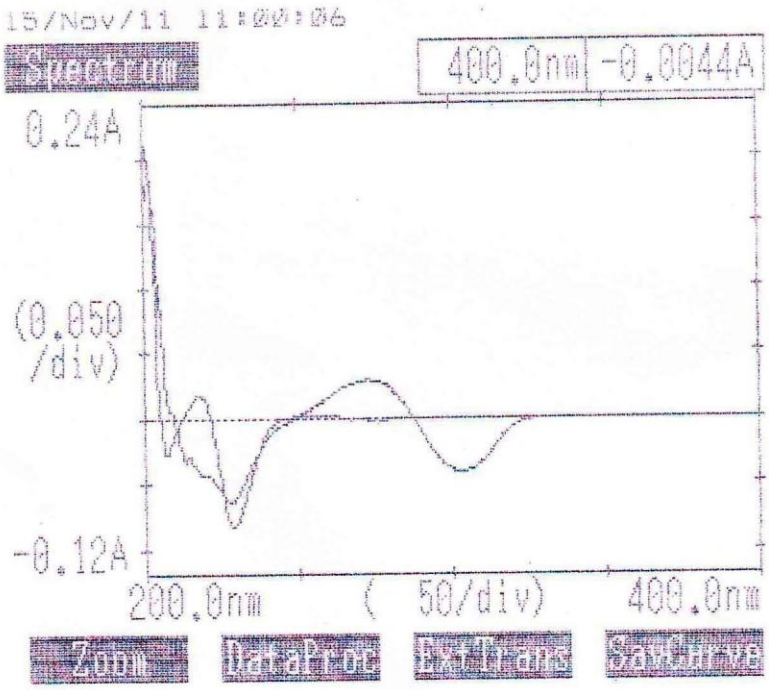


Fig - 13

CALIBRATION CURVE OF IBUPROFEN IN METHANOL AT 209.5 nm

(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

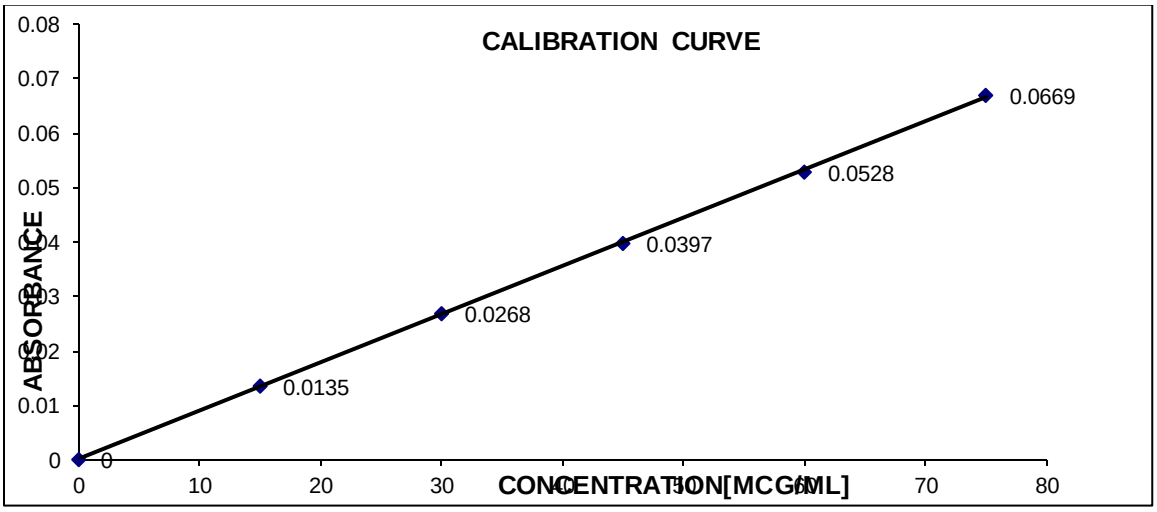


Fig - 14

CALIBRATION CURVE OF FAMOTIDINE IN METHANOL AT 248.0 nm

(FIRST ORDER DERIVATIVE SPECTROPHOTOMETRIC METHOD)

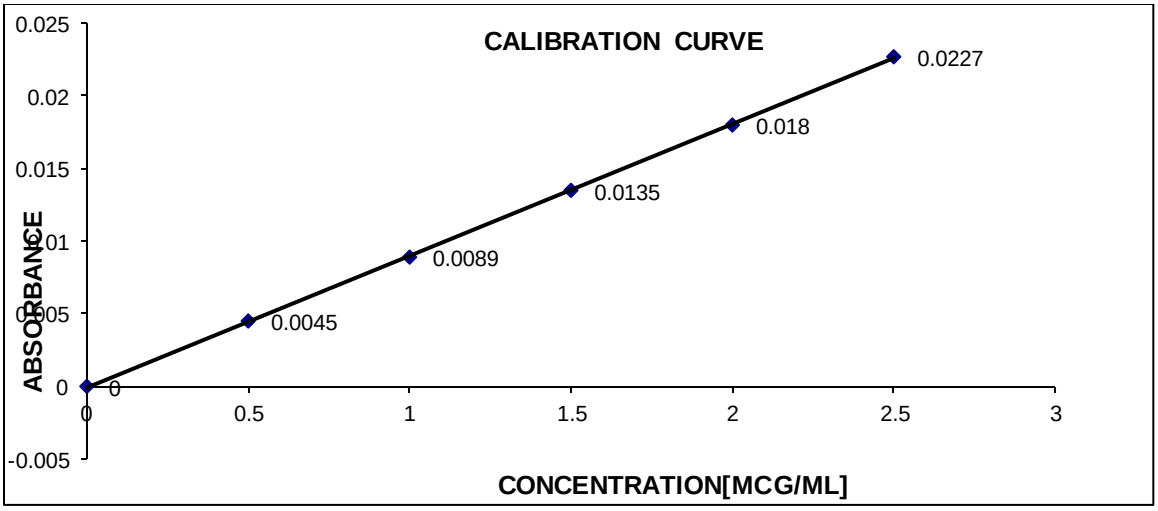
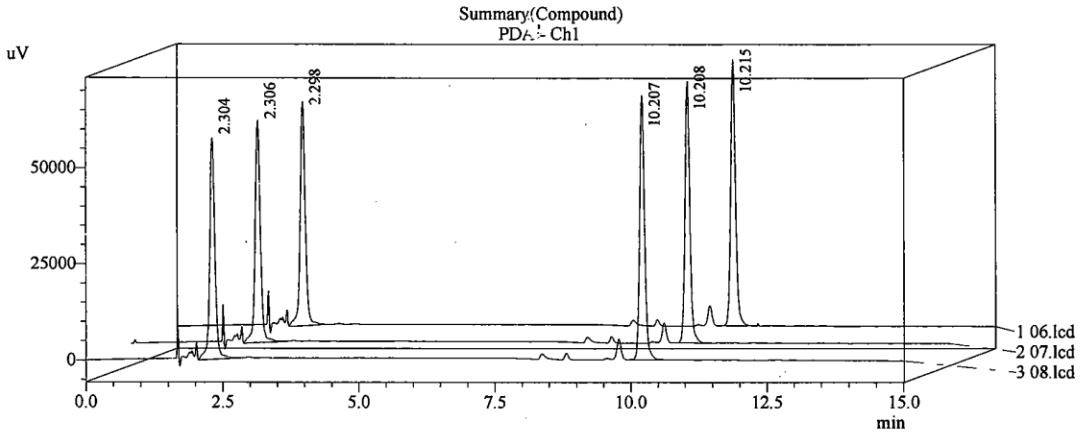


Fig – 15

LINEARITY CHROMATOGRAM OF IBUPROFEN AND FAMOTIDINE (300, 10 MCG/ML)

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

SUMMARY REPORT



<< PDA >>

ID#1 Compound Name: Famotidine

Original Data File	ate Acquire	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\06.lcd	2/18/2011	5:11:35 PM	Linearity 1	2.298	388199	2576	1.080	0.000	0.000
E:\Mpl\Project\07.lcd	2/18/2011	5:27:04 PM	Linearity 1	2.306	388797	2478	1.090	0.000	0.000
E:\Mpl\Project\08.lcd	2/18/2011	5:42:31 PM	Linearity 1	2.304	387490	2472	1.073	0.000	0.000
				2.303	388162	2509	1.081	0.000	0.000
				0.173	0.169	2.332	0.808	0.000	0.000

ID#2 Compound Name: Ibuprofen

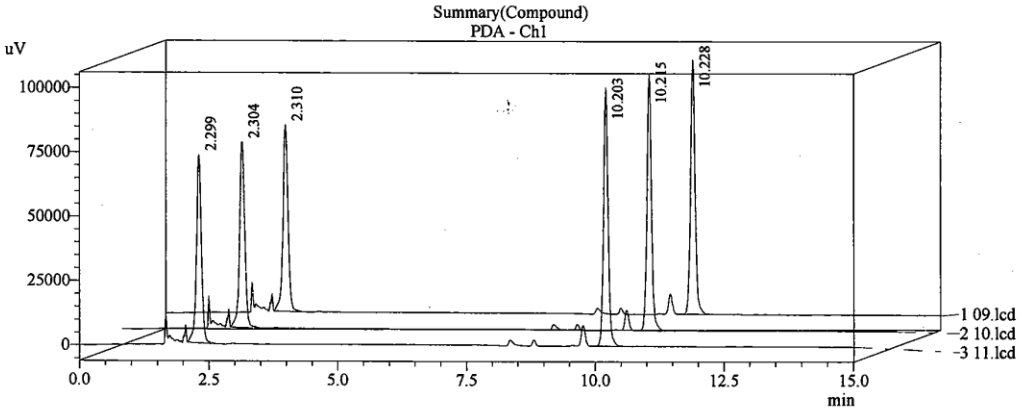
Original Data File	ate Acquire	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\06.lcd	2/18/2011	5:11:35 PM	Linearity 1	10.215	422059	53444	1.131	44.243	3.445
E:\Mpl\Project\07.lcd	2/18/2011	5:27:04 PM	Linearity 1	10.208	419808	55188	1.134	44.010	3.427
E:\Mpl\Project\08.lcd	2/18/2011	5:42:31 PM	Linearity 1	10.207	421406	53771	1.127	43.727	3.430
				10.210	421091	54134	1.131	43.993	3.434
				0.044	0.275	1.713	0.298	0.587	0.277

Fig –16

LINEARITY CHROMATOGRAM OF IBUPROFEN AND FAMOTIDINE (450, 15 MCG/ML)

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

SUMMARY REPORT



<< PDA >>

ID#1 Compound Name: Famotidine

Original Data File	ate Acquire	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\09.lcd	2/18/2011	5:57:58 PM	Linearity 2	2.310	495890	2311	1.015	0.000	0.000
E:\Mpl\Project\10.lcd	2/18/2011	6:13:27 PM	Linearity 2	2.304	495818	2319	1.012	0.000	0.000
E:\Mpl\Project\11.lcd	2/18/2011	6:28:56 PM	Linearity 2	2.299	490789	2359	0.993	0.000	0.000
				2.305	494166	2330	1.007	0.000	0.000
				0.240	0.592	1.119	1.148	0.000	0.000

ID#2 Compound Name: Ibuprofen

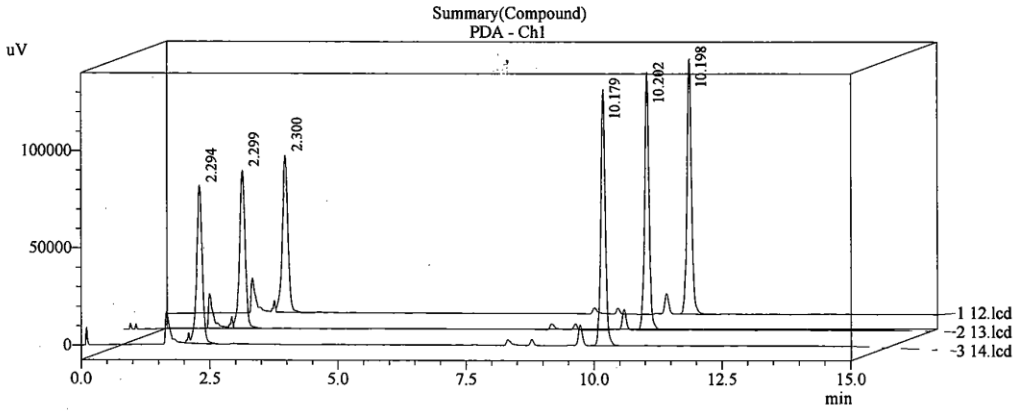
Original Data File	ate Acquire	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\09.lcd	2/18/2011	5:57:58 PM	Linearity 2	10.228	602320	54589	1.079	43.105	3.427
E:\Mpl\Project\10.lcd	2/18/2011	6:13:27 PM	Linearity 2	10.215	600970	53650	1.074	43.021	3.434
E:\Mpl\Project\11.lcd	2/18/2011	6:28:56 PM	Linearity 2	10.203	604495	53991	1.068	43.310	3.438
				10.215	602595	54076	1.074	43.145	3.433
				0.119	0.295	0.879	0.528	0.345	0.157

Fig –17

LINEARITY CHROMATOGRAM OF IBUPROFEN AND FAMOTIDINE (600, 20 MCG/ML)

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

SUMMARY REPORT



<< PDA >>

ID#1 Compound Name: Famotidine

Original Data File	ate Acquir	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\12.lcd	2/18/2011	6:44:24 PM	Linearity 3	2.300	606460	1934	0.937	0.000	0.000
E:\Mpl\Project\13.lcd	2/18/2011	6:59:53 PM	Linearity 3	2.299	605680	1959	0.938	0.000	0.000
E:\Mpl\Project\14.lcd	2/18/2011	7:15:22 PM	Linearity 3	2.294	622088	1964	0.949	0.000	0.000
				2.298	611409	1952	0.941	0.000	0.000
				0.129	1.514	0.840	0.664	0.000	0.000

ID#2 Compound Name: Ibuprofen

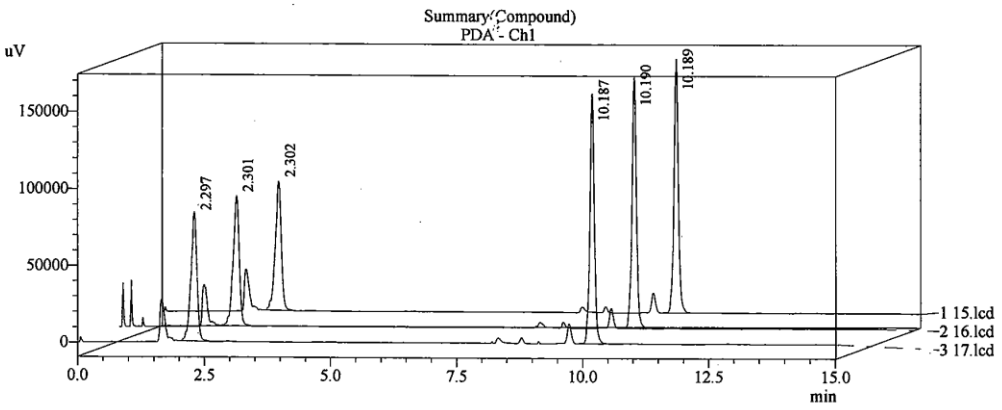
Original Data File	ate Acquir	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\12.lcd	2/18/2011	6:44:24 PM	Linearity 3	10.198	795535	57973	1.030	41.723	3.435
E:\Mpl\Project\13.lcd	2/18/2011	6:59:53 PM	Linearity 3	10.202	794715	57798	1.019	41.865	3.437
E:\Mpl\Project\14.lcd	2/18/2011	7:15:22 PM	Linearity 3	10.179	793247	57866	1.015	41.905	3.437
				10.193	794499	57879	1.021	41.831	3.436
				0.119	0.146	0.152	0.791	0.228	0.040

Fig –18

LINEARITY CHROMATOGRAM OF IBUPROFEN AND FAMOTIDINE (750, 25 MCG/ML)

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

SUMMARY REPORT



<< PDA >>

ID#1 Compound Name: Famotidine

Original Data File	ate Acquir	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\15.lcd	2/18/2011	7:30:51 PM	Linearity 4	2.302	712070	1483	0.852	0.000	0.000
E:\Mpl\Project\16.lcd	2/18/2011	7:46:20 PM	Linearity 4	2.301	712325	1491	0.856	0.000	0.000
E:\Mpl\Project\17.lcd	2/18/2011	8:01:49 PM	Linearity 4	2.297	708583	1557	0.860	0.000	0.000
				2.300	710993	1510	0.856	0.000	0.000
				0.099	0.294	2.676	0.509	0.000	0.000

ID#2 Compound Name: Ibuprofen

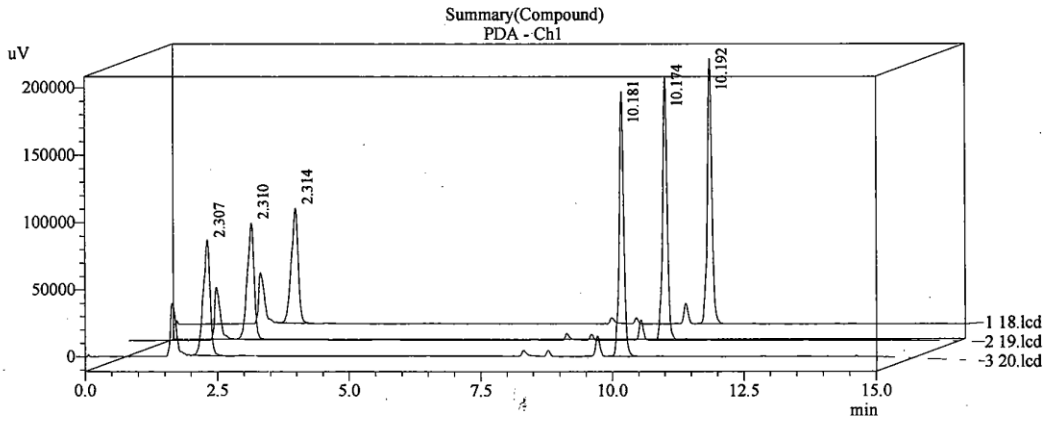
Original Data File	ate Acquir	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\15.lcd	2/18/2011	7:30:51 PM	Linearity 4	10.189	985632	59091	0.997	38.787	3.427
E:\Mpl\Project\16.lcd	2/18/2011	7:46:20 PM	Linearity 4	10.190	987128	58492	0.985	38.776	3.429
E:\Mpl\Project\17.lcd	2/18/2011	8:01:49 PM	Linearity 4	10.187	985119	58925	0.983	39.372	3.434
				10.189	985960	58836	0.988	38.978	3.430
				0.014	0.106	0.525	0.782	0.875	0.111

Fig –19

LINEARITY CHROMATOGRAM OF IBUPROFEN AND FAMOTIDINE (900, 30 MCG/ML)

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

SUMMARY REPORT



<< PDA >>

ID#1 Compound Name: Famotidine

Original Data File	Date Acquired	Time Acquired	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\18.lcd	2/18/2011	8:17:19 PM	Linearity 5	2.314	811214	1196	0.840	0.000	0.000
E:\Mpl\Project\19.lcd	2/18/2011	8:32:50 PM	Linearity 5	2.310	817456	1187	0.849	0.000	0.000
E:\Mpl\Project\20.lcd	2/18/2011	8:48:21 PM	Linearity 5	2.307	805731	1236	0.852	0.000	0.000
				2.310	811467	1207	0.847	0.000	0.000
				0.157	0.723	2.179	0.771	0.000	0.000

ID#2 Compound Name: Ibuprofen

Original Data File	ate Acquired	Time Acquired	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\18.lcd	2/18/2011	8:17:19 PM	Linearity 5	10.192	1178780	55890	0.964	35.802	3.405
E:\Mpl\Project\19.lcd	2/18/2011	8:32:50 PM	Linearity 5	10.174	1179263	58030	0.961	35.976	3.403
E:\Mpl\Project\20.lcd	2/18/2011	8:48:21 PM	Linearity 5	10.181	1179429	59342	0.960	36.659	3.413
				10.182	1179157	57754	0.961	36.146	3.407
				0.092	0.029	3.017	0.214	1.253	0.159

Fig –20

CALIBRATION CURVE OF IBUPROFEN BY RP-HPLC

MEDOPHARM PRIVATE LIMITED
GUDUVANCHERY
ANALYTICAL METHOD VALIDATION CALCULATIONS - LINEARITY

Conc (mcg)	Ibuprofen
300	421091
450	602595
600	794499
750	985960
900	1179157

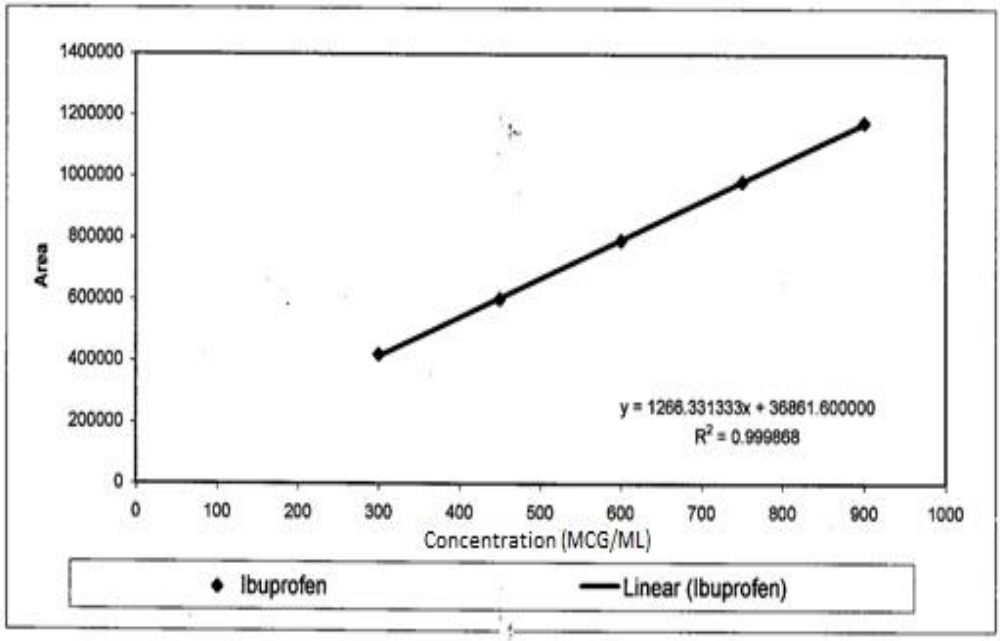


Fig –21

CALIBRATION CURVE OF FAMOTIDINE BY RP-HPLC

MEDOPHARM PRIVATE LIMITED
GUDUVANCHERY
ANALYTICAL METHOD VALIDATION CALCULATIONS - LINEARITY

Conc (mcg)	Famotidine
10	388162
15	494166
20	611409
25	710993
30	811467

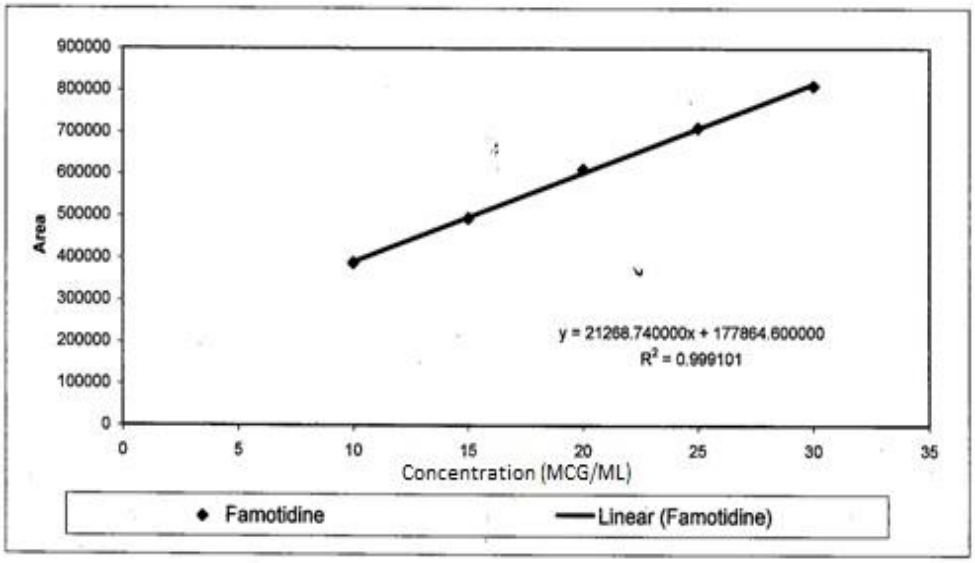
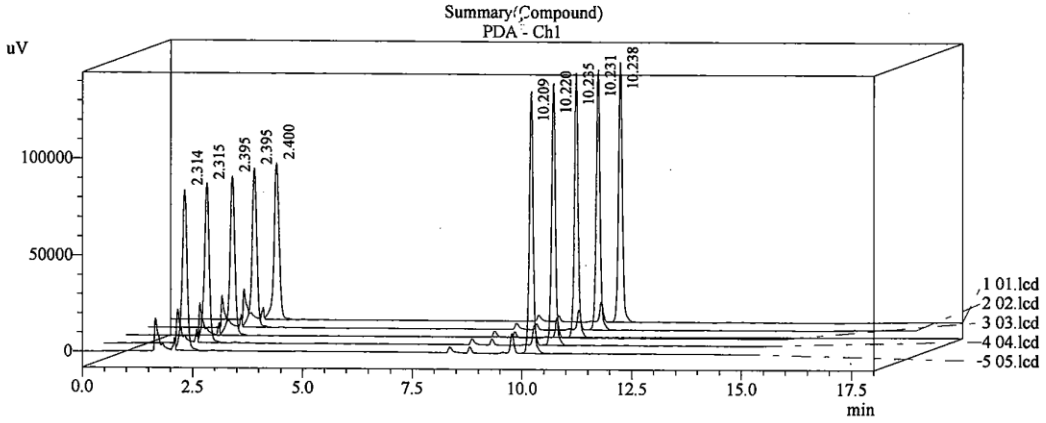


Fig –22

STANDARD CHROMATOGRAM OF IBUPROFEN AND FAMOTIDINE

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

SUMMARY REPORT



<< PDA >>

ID#1 Compound Name: Famotidine

Original Data File	ate Acquire	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\01.lcd	2/18/2011	3:41:11 PM	Std 1	2.400	648242	1988	0.931	0.000	0.000
E:\Mpl\Project\02.lcd	2/18/2011	3:59:39 PM	Std 2	2.395	647479	2026	0.936	0.000	0.000
E:\Mpl\Project\03.lcd	2/18/2011	4:25:11 PM	Std 3	2.395	653556	2025	0.926	0.000	0.000
E:\Mpl\Project\04.lcd	2/18/2011	4:40:39 PM	Std 4	2.315	642938	1857	0.941	0.000	0.000
E:\Mpl\Project\05.lcd	2/18/2011	4:56:08 PM	Std 5	2.314	648181	1858	0.944	0.000	0.000
				2.364	648079	1951	0.936	0.000	0.000
				1.902	0.582	4.443	0.795	0.000	0.000

ID#2 Compound Name: Ibuprofen

Original Data File	ate Acquire	Time Acquire	Sample ID	Ret. Time	Area	oretical Pl	ailing Fact	Resolution	k'
E:\Mpl\Project\01.lcd	2/18/2011	3:41:11 PM	Std 1	10.238	810753	55096	1.112	40.210	3.265
E:\Mpl\Project\02.lcd	2/18/2011	3:59:39 PM	Std 2	10.231	813556	57180	1.094	40.819	3.272
E:\Mpl\Project\03.lcd	2/18/2011	4:25:11 PM	Std 3	10.235	827250	53979	1.083	40.296	3.273
E:\Mpl\Project\04.lcd	2/18/2011	4:40:39 PM	Std 4	10.220	824659	57115	1.066	40.959	3.414
E:\Mpl\Project\05.lcd	2/18/2011	4:56:08 PM	Std 5	10.209	826837	56897	1.058	40.907	3.411
				10.227	820611	56053	1.083	40.638	3.327
				0.118	0.956	2.574	2.004	0.877	2.347

Fig –23

CHROMATOGRAM FOR ANALYSIS OF FORMULATION

REPEATABILITY – 1

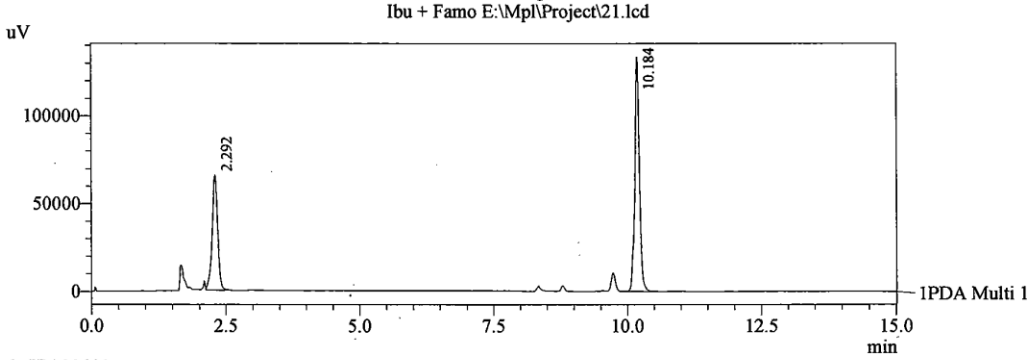
MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information
Acquired by : Admin
Sample Name : Ibu + Famo
Sample ID : Precision 1
Tray# : 1
Vial# : 7
Injection Volume : 20
Method File : E:\Mpl\Project\Ibufamo.lcm
Data File : E:\Mpl\Project\21.lcd
Batch File : E:\Mpl\Project\Ibufamo.lcb
Acquired time : 12/18/2011, 9:03:50 PM

<<Peak Integration>>
<PDA>
Channel :Extracted Chrom
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Channel :Ch1 265nm
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Chromatogram
Ibu + Famo E:\Mpl\Project\21.lcd



1 PDA Multi 1

PeakTable E:\Mpl\Project\21.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Plat	Tailing Factor	k'
1	Famotidine	2.292	475840	65584	37.076	0.000	2028.3	0.951	0.000
2	Ibuprofen	10.184	807587	133409	62.924	42.102	56536.5	0.990	3.443
Total			1283427		100.000				

Fig –24

CHROMATOGRAM FOR ANALYSIS OF FORMULATION

REPEATABILITY – 2

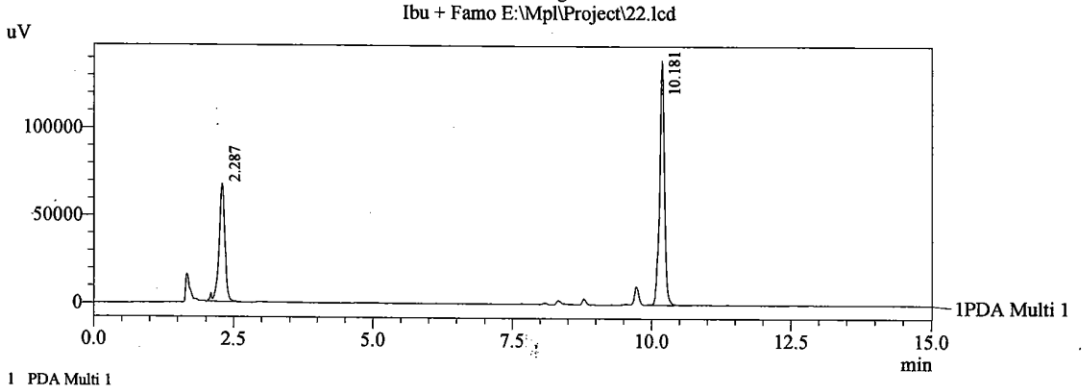
MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information	
Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Precision 2
Tray#	: 1
Vial#	: 8
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\22.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/18/2011, 9:19:21 PM

<<Peak Integration>>
<PDA>

Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area
Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area

Chromatogram
Ibu + Famo E:\Mpl\Project\22.lcd



1 PDA Multi 1

PeakTable E:\Mpl\Project\22.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Pla	Tailing Factor	k'
1	Famotidine	2.287	497664	67584	37.185	0.000	2135.9	0.939	0.000
2	Ibuprofen	10.181	840681	139186	62.815	43.584	61402.8	0.980	3.452
Total			1338345		100.000				

Fig –25

CHROMATOGRAM FOR ANALYSIS OF FORMULATION

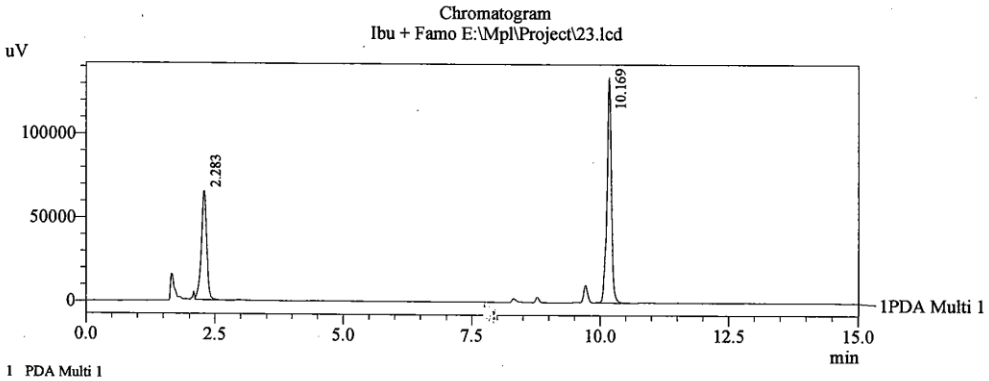
REPEATABILITY – 3

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information
Acquired by : Admin
Sample Name : Ibu + Famo
Sample ID : Precision 3
Tray# : 1
Vial# : 9
Injection Volume : 20
Method File : E:\Mpl\Project\Ibufamo.lcm
Data File : E:\Mpl\Project\23.lcd
Batch File : E:\Mpl\Project\Ibufamo.lcb
Acquired time : 12/18/2011, 9:34:51 PM

<<Peak Integration>>
<PDA>
Channel :Extracted Chrom
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Channel :Ch1 265nm
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area



1 PDA Multi 1

PeakTable E:\Mpl\Project\23.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	Theoretical Plat	Tailing Factor	k'
1	Famotidine	2.283	471728	65150	36.672	0.000	2024.2	0.941	0.000
2	Ibuprofen	10.169	814625	134464	63.328	42.335	57556.8	0.983	3.454
Total			1286353		100.000				

Fig –26

CHROMATOGRAM FOR ANALYSIS OF FORMULATION

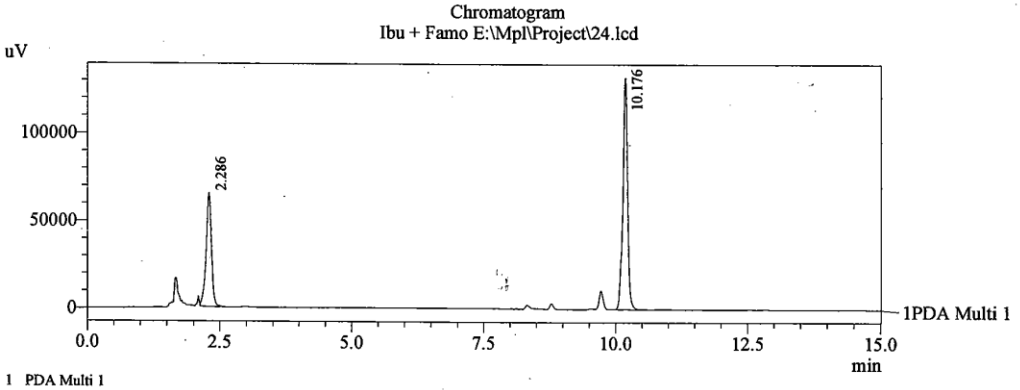
REPEATABILITY – 4

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information
Acquired by : Admin
Sample Name : Ibu + Famo
Sample ID : Precision 4
Tray# : 1
Vial# : 10
Injection Volume : 20
Method File : E:\Mpl\Project\Ibufamo.lcm
Data File : E:\Mpl\Project\24.lcd
Batch File : E:\Mpl\Project\Ibufamo.lcb
Acquired time : 12/18/2011, 9:50:21 PM

<<Peak Integration>>
<PDA>
Channel :Extracted Chrom
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Channel :Ch1 265nm
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area



1 PDA Multi 1

PeakTable E:\Mpl\Project\24.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Pla	Tailing Factor	k'
1	Famotidine	2.286	467126	65062	36.657	0.000	2125.7	0.939	0.000
2	Ibuprofen	10.176	807199	131817	63.343	42.923	57763.5	0.984	3.452
Total			1274325		100.000				

Fig –27

CHROMATOGRAM FOR ANALYSIS OF FORMULATION

REPEATABILITY – 5

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information

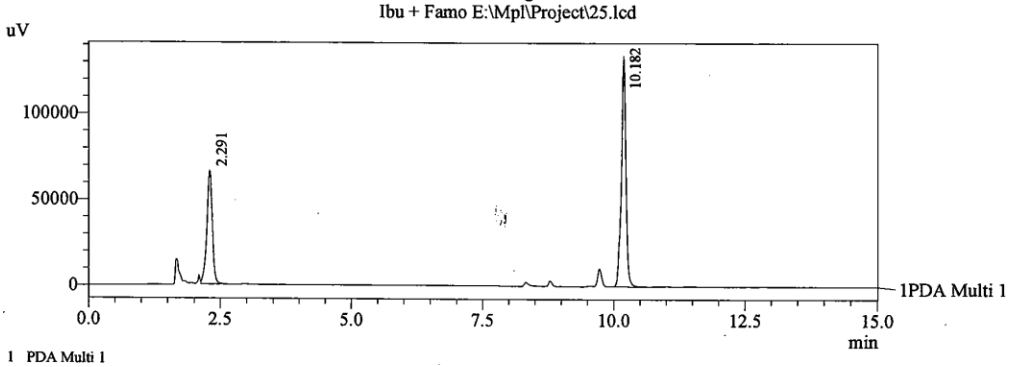
Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Precision 5
Tray#	: 1
Vial#	: 11
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\25.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/18/2011, 10:05:51 PM

<<Peak Integration>>
<PDA>

Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area

Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area

Chromatogram
Ibu + Famo E:\Mpl\Project\25.lcd



1 PDA Multi 1

PeakTable E:\Mpl\Project\25.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	Theoretical Plat	Tailing Factor	k'
1	Famotidine	2.291	476634	66303	37.014	0.000	2051.6	0.949	0.000
2	Ibuprofen	10.182	811084	133808	62.986	41.789	53949.5	0.979	3.444
Total			1287718		100.000				

Fig –28

CHROMATOGRAM FOR ANALYSIS OF FORMULATION

REPEATABILITY – 6

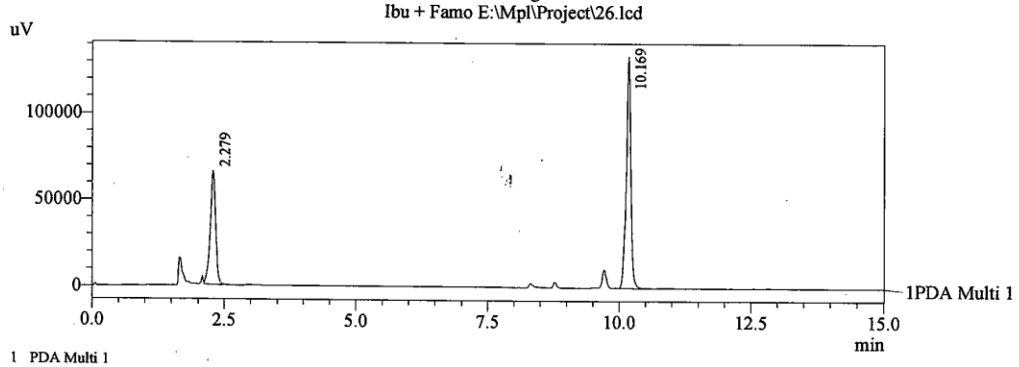
MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information
Acquired by : Admin
Sample Name : Ibu + Famo
Sample ID : Precision 6
Tray# : 1
Vial# : 12
Injection Volume : 20
Method File : E:\Mpl\Project\Ibufamo.lcm
Data File : E:\Mpl\Project\26.lcd
Batch File : E:\Mpl\Project\Ibufamo.lcb
Acquired time : 12/18/2011, 10:21:22 PM

<<Peak Integration>>
<PDA>
Channel :Extracted Chrom
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Channel :Ch1 265nm
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Chromatogram
Ibu + Famo E:\Mpl\Project\26.lcd



1 PDA Multi 1

PeakTable E:\Mpl\Project\26.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	Theoretical Plat	Tailing Factor	k'
1	Famotidine	2.279	469528	65793	36.662	0.000	2050.4	0.942	0.000
2	Ibuprofen	10.169	811181	133448	63.338	42.523	57439.5	0.978	3.461
Total			1280709		100.000				

Fig –29

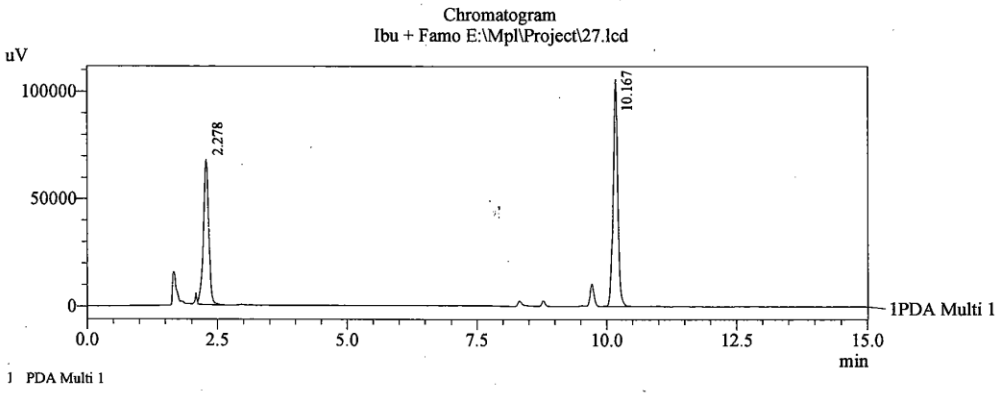
CHROMATOGRAM FOR 80 % RECOVERY OF FORMULATION

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information	
Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Accuracy 1
Tray#	: 1
Vial#	: 13
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\27.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/18/2011, 10:36:51 PM

<<Peak Integration>>
<PDA>

Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area
Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area



1 PDA Multi 1

PeakTable E:\Mpl\Project\27.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	Theoretical Plat	Tailing Factor	k'
1	Famotidine	2.278	481931	67549	42.530	0.000	2034.8	0.944	0.000
2	Ibuprofen	10.167	651216	105554	57.470	42.167	55779.9	1.006	3.463
Total			1133147		100.000				

Fig –30

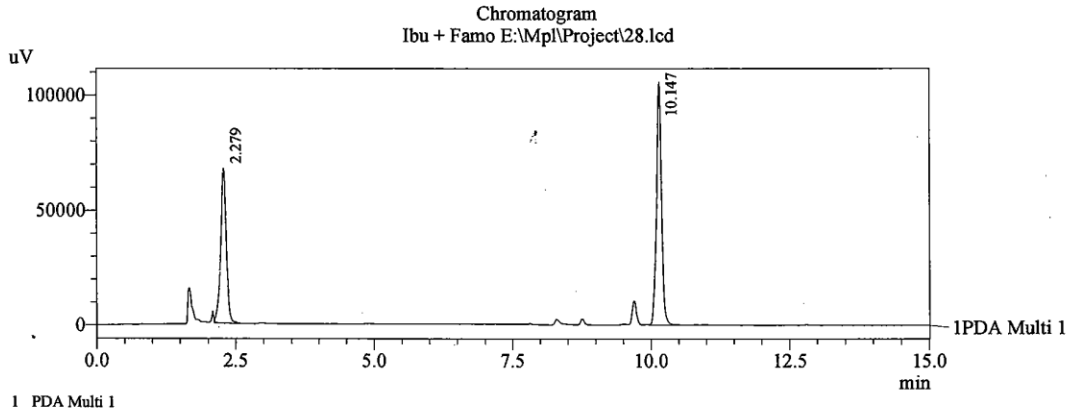
CHROMATOGRAM FOR 80 % RECOVERY OF FORMULATION

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information	
Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Accuracy 2
Tray#	: 1
Vial#	: 14
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\28.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/18/2011, 10:52:18 PM

<<Peak Integration>>
<PDA>

Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area
Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area



1 PDA Multi 1

PeakTable E:\Mpl\Project\28.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Plat	Tailing Factor	k'
1	Famotidine	2.279	482596	67651	42.592	0.000	2044.8	0.945	0.000
2	Ibuprofen	10.147	650477	105459	57.408	42.026	55129.9	1.003	3.453
Total			1133073		100.000				

Fig –31

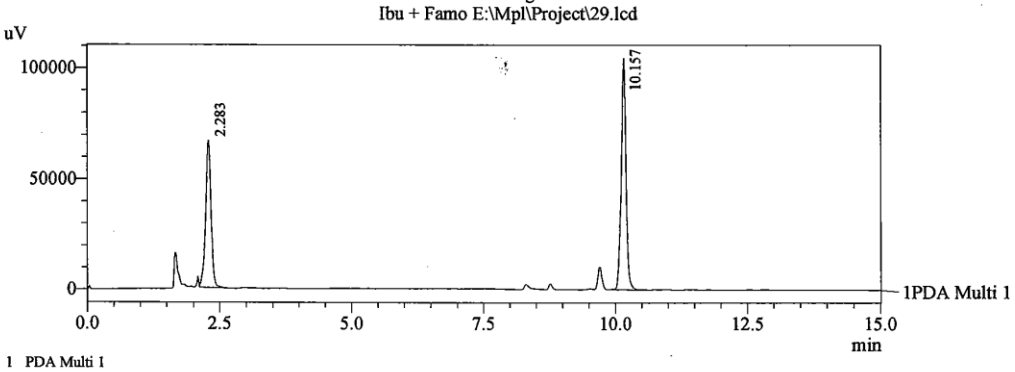
CHROMATOGRAM FOR 80 % RECOVERY OF FORMULATION

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information	
Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Accuracy 3
Tray#	: 1
Vial#	: 15
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\29.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/18/2011, 11:07:49 PM

<<Peak Integration>>	
<PDA>	
Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area
Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area

Chromatogram
Ibu + Famo E:\Mpl\Project\29.lcd



1 PDA Multi 1

PeakTable E:\Mpl\Project\29.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Pla	Tailing Factor	k'
1	Famotidine	2.283	479399	66791	42.571	0.000	2059.9	0.948	0.000
2	Ibuprofen	10.157	646705	104475	57.429	42.087	55195.8	1.005	3.448
Total			1126104		100.000				

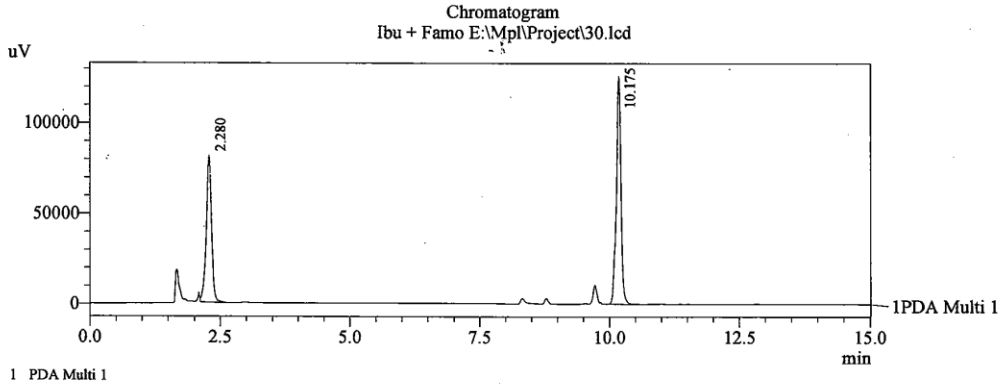
Fig –32

CHROMATOGRAM FOR 100 % RECOVERY OF FORMULATION

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information	
Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Accuracy 4
Tray#	: 1
Vial#	: 16
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\30.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/18/2011, 11:23:18 PM

<<Peak Integration>>	
<PDA>	
Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area
Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area



1 PDA Multi 1

PeakTable E:\Mpl\Project\30.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	Theoretical Plat	Tailing Factor	k'
1	Famotidine	2.280	579953	81176	42.780	0.000	2049.3	0.941	0.000
2	Ibuprofen	10.175	775724	125877	57.220	42.446	56927.8	0.983	3.463
Total			1355677		100.000				

Fig –33

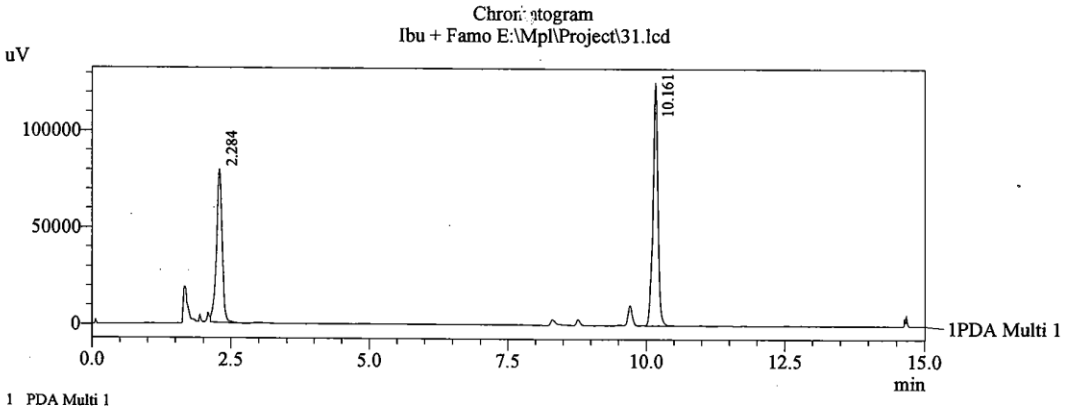
CHROMATOGRAM FOR 100 % RECOVERY OF FORMULATION

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information
Acquired by : Admin
Sample Name : Ibu + Famo
Sample ID : Accuracy 5
Tray# : 1
Vial# : 17
Injection Volume : 20
Method File : E:\Mpl\Project\Ibufamo.lcm
Data File : E:\Mpl\Project\31.lcd
Batch File : E:\Mpl\Project\Ibufamo.lcb
Acquired time : 12/18/2011, 11:38:49 PM

<<Peak Integration>>
<PDA>
Channel :Extracted Chrom
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Channel :Ch1 265nm
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area



1 PDA Multi 1

PeakTable E:\Mpl\Project\31.lcd
PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Plat	Tailing Factor	k'
1	Famotidine	2.284	566170	79150	42.474	0.000	2060.8	0.930	0.000
2	Ibuprofen	10.161	766809	125413	57.526	41.638	52667.5	0.979	3.449
Total			1332979		100.000				

Fig –34

CHROMATOGRAM FOR 100 % RECOVERY OF FORMULATION

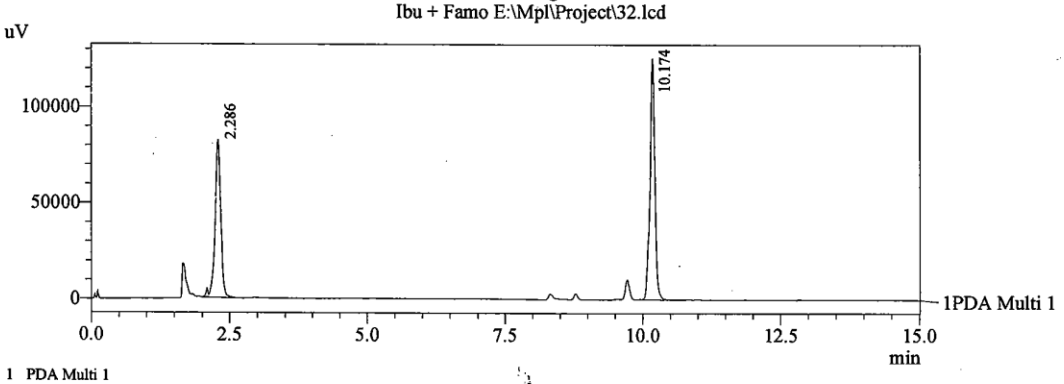
MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information
Acquired by : Admin
Sample Name : Ibu + Famo
Sample ID : Accuracy 6
Tray# : 1
Vial# : 18
Injection Volume : 20
Method File : E:\Mpl\Project\Ibufamo.lcm
Data File : E:\Mpl\Project\32.lcd
Batch File : E:\Mpl\Project\Ibufamo.lcb
Acquired time : 12/18/2011, 11:54:20 PM

<<Peak Integration>>
<PDA>
Channel :Extracted Chrom
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Channel :Ch1 265nm
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Chromatogram
Ibu + Famo E:\Mpl\Project\32.lcd



1 PDA Multi 1

PeakTable E:\Mpl\Project\32.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	eoretical Plat	Tailing Factor	k'
1	Famotidine	2.286	595571	82075	43.613	0.000	2173.2	0.947	0.000
2	Ibuprofen	10.174	770010	125396	56.387	42.730	55342.4	0.981	3.450
Total			1365581		100.000				

Fig –35

CHROMATOGRAM FOR 120 % RECOVERY OF FORMULATION

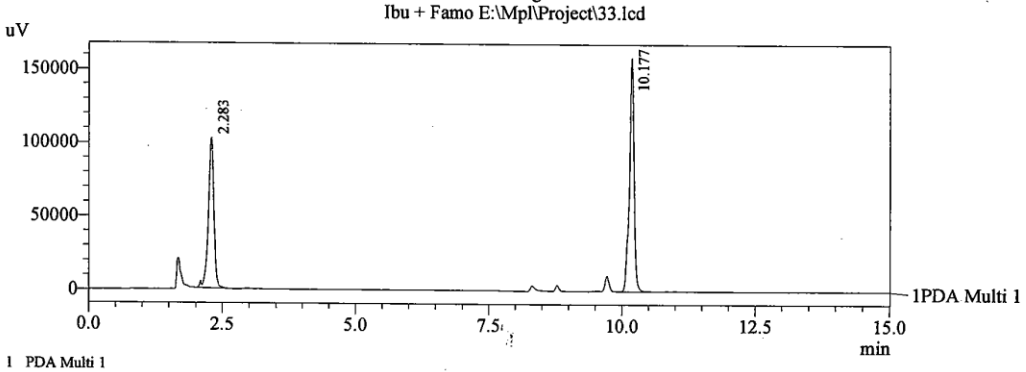
MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information	
Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Accuracy 7
Tray#	: 1
Vial#	: 19
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\33.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/19/2011, 12:09:49 AM

<<Peak Integration>>
<PDA>

Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area
Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area

Chromatogram
Ibu + Famo E:\Mpl\Project\33.lcd



1 PDA Multi 1

PeakTable E:\Mpl\Project\33.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	Theoretical Plat	Tailing Factor	k'
1	Famotidine	2.283	744229	102232	43.487	0.000	2068.9	0.944	0.000
2	Ibuprofen	10.177	967139	158683	56.513	42.856	58949.9	0.953	3.458
Total			1711368		100.000				

Fig –36

CHROMATOGRAM FOR 120 % RECOVERY OF FORMULATION

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

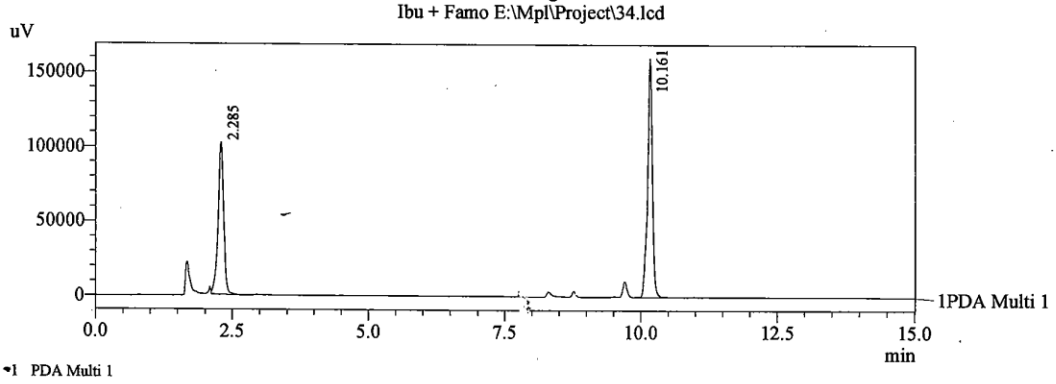
Sample Information

Acquired by	: Admin
Sample Name	: Ibu + Famo
Sample ID	: Accuracy 8
Tray#	: 1
Vial#	: 20
Injection Volume	: 20
Method File	: E:\Mpl\Project\Ibufamo.lcm
Data File	: E:\Mpl\Project\34.lcd
Batch File	: E:\Mpl\Project\Ibufamo.lcb
Acquired time	: 12/19/2011, 12:25:22 AM

<<Peak Integration>>
<PDA>

Channel	:Extracted Chrom
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area
Channel	:Ch1 265nm
Width	:5 sec
Slope	:1000 uV/min
Min Area/Height	:1000 counts
Calculated by	:Area

Chromatogram
Ibu + Famo E:\Mpl\Project\34.lcd



•1 PDA Multi 1

PeakTable E:\Mpl\Project\34.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Plat	Tailing Factor	k'
1	Famotidine	2.285	726159	102021	42.869	0.000	2093.7	0.936	0.000
2	Ibuprofen	10.161	967756	159746	57.131	42.631	57330.7	0.950	3.447
Total			1693915		100.000				

Fig –37

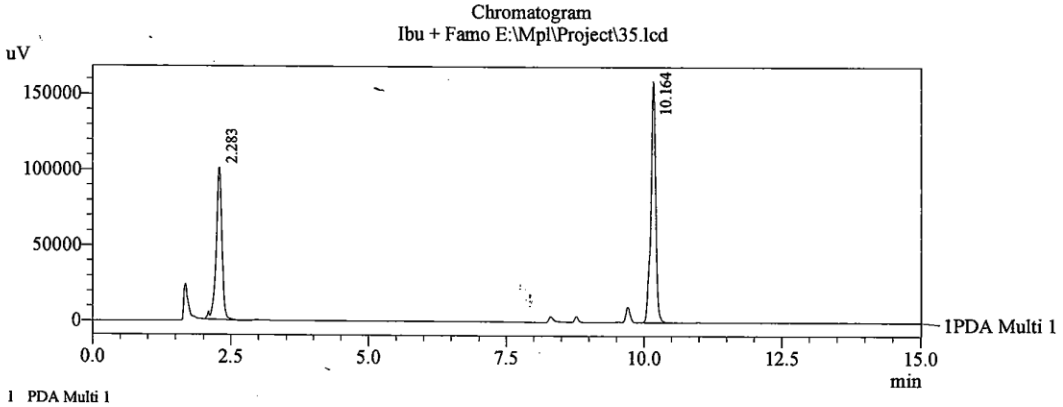
CHROMATOGRAM FOR 120 % RECOVERY OF FORMULATION

MEDOPHARM PRIVATE LIMITED - QUALITY CONTROL DEPARTMENT

Sample Information
Acquired by : Admin
Sample Name : Ibu + Famo
Sample ID : Accuracy 9
Tray# : 1
Vial# : 21
Injection Volume : 20
Method File : E:\Mpl\Project\Ibufamo.lcm
Data File : E:\Mpl\Project\35.lcd
Batch File : E:\Mpl\Project\Ibufamo.lcb
Acquired time : 12/19/2011, 12:40:50 AM

<<Peak Integration>>
<PDA>
Channel :Extracted Chrom
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area

Channel :Ch1 265nm
Width :5 sec
Slope :1000 uV/min
Min Area/Height :1000 counts
Calculated by :Area



1 PDA Multi 1

PeakTable E:\Mpl\Project\35.lcd

PDA Ch1 265nm 4nm

Peak#	Name	Ret. Time	Area	Height	Area %	Resolution	heoretical Plat	Tailing Factor	k'
1	Famotidine	2.283	733206	100732	42.974	0.000	2074.7	0.928	0.000
2	Ibuprofen	10.164	972939	159350	57.026	42.774	58565.9	0.947	3.452
Total			1706145		100.000				

TABLES

TABLE-1

SOLUBILITY PROFILE OF IBUPROFEN AND FAMOTIDINE

S.No.	SOLVENTS	IBUPROFEN	FAMOTIDINE
1.	Acetonitrile	Slightly soluble	Practically soluble
2.	Acetone	Slightly soluble	Insoluble
3.	Chloroform	Practically soluble	Slightly soluble
4.	Dimethyl formamide	Slightly soluble	Soluble
5.	Dichloromethane	Soluble	Insoluble
6.	Petrolieum ether	Insoluble	Insoluble
7.	Ethanol	Slightly soluble	Practically soluble
8.	Glacial acetic acid	Soluble	Soluble
9.	HCl (0.1N)	Insoluble	Practically soluble
10.	Methanol	Soluble	Soluble
11.	NaOH (0.1N)	Practically soluble	Insoluble
12.	Water	Slightly soluble	Slightly soluble
13.	Benzene	Slightly soluble	Practically soluble
14.	n-Butanol	Insoluble	Insoluble
15.	Buffer pH-7	Soluble	Slightly soluble

TABLE-2

OPTICAL PARAMETERS OF IBUPROFEN AND FAMOTIDINE (DERIVATIVE METHOD)

PARAMETERS	IBUPROFEN	FAMOTIDINE
λ_{max}(nm)	209.5	248
Beers law limit (µg/mL)	15-75	0.5-2.5
Sandell's sensitivity (µg/cm^2/0.001 A.U)	1.12611	0.11494
Molar absorptivity (L mol^{-1} cm^{-1})	189.875	2943.37
Correlation coefficient (r)	0.9999	0.9999
Regressionequation (y = mx + c)	Y = 0.0008797 X + (0.0004666)	Y = 0.008719 X + (0.00002533)
Slope(m)	0.0008797	0.008719
Intercept(c)	0.0004666	0.00002533
LOD (µg/mL)	0.9613	0.0450
LOQ (µg/mL)	2.9132	0.1335
Standard error of mean of Regression line	0.0005038	0.0001117

TABLE-3

OPTICAL PARAMETERS OF IBUPROFEN

(AREA UNDER THE CURVE METHOD)

PARAMETERS	IBUPROFEN	IBUPROFEN
λ_{max}(nm)	227.5 220.0	294.0 282.0
Beers law limit (µg/mL)	15-75	0.5-2.5
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.00335	0.23411
Molar absorptivity (L mol^{-1} cm^{-1})	63821.32	896.569
Correlation coefficient (r)	0.9999	0.9999
Regressionequation (y = mx + c)	Y = 0.2980 X + (0.1287)	Y = 0.0045 X + (0.0028)
Slope(m)	0.2980	0.0045
Intercept(c)	0.1287	0.0028
LOD (µg/mL)	1.4393	0.0751
LOQ (µg/mL)	4.3615	0.1693
Standard error of mean of Regression line	0.13386	0.00223

TABLE-4

OPTICAL PARAMETERS OF FAMOTIDINE

(AREA UNDER CURVE METHOD)

PARAMETERS	FAMOTIDINE	FAMOTIDINE
λ_{max}(nm)	227.5 220.0	294.0 282.0
Beers law limit (µg/mL)	15-75	0.5-2.5
Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.002174	0.001025
Molar absorptivity (L mol^{-1} cm^{-1})	61283.23	869.695
Correlation coefficient (r)	0.9999	0.9999
Regressionequation (y = mx + c)	Y = 0.4600 X + (0.1171)	Y = 0.9769 X + (0.0146)
Slope(m)	0.4600	0.9769
Intercept(c)	0.1171	0.0146
LOD (µg/mL)	0.0225	0.0400
LOQ (µg/mL)	0.0684	0.1213
Standard error of mean of Regression line	0.00813	0.01673

TABLE-5

ASSAY OF TABLET FORMULATION BY UV- SPECTROSCOPY

(AREA UNDER THE CURVEAND SIMULTANEOUS EQUATION METHOD)

Drug	S.No	Labeled amount (mg/tab)	Amount found (mg)	Percentage obtained	Average	S.D.	% RSD	S.E
IB	1	800	801.04	100.13	100.01	0.12247	0.12246	0.0034
	2	800	799.74	99.96				
	3	800	801.04	100.13				
	4	800	798.69	99.83				
	5	800	799.46	99.93				
	6	800	800.64	100.08				
FA	1	26.60	26.65	100.20	100.14	0.0751	0.0750	0.0020
	2	26.60	26.66	100.25				
	3	26.60	26.62	100.08				
	4	26.60	26.64	100.16				
	5	26.60	26.61	100.07				
	6	26.60	26.62	100.08				

TABLE-6

ASSAY OF TABLET FORMULATION BY UV- SPECTROSCOPY (DERIVATIVE SPECTRA METHOD)

Drug	S.No	Labeled amount (mg/tab)	Amount found(mg)	Percentage obtained	Average	S.D.	% RSD	S.E.
IB	1	800	801.04	100.13	100.13	0.4989	0.4982	0.0138
	2	800	806.90	100.86				
	3	800	803.97	100.49				
	4	800	797.85	99.73				
	5	800	795.91	99.48				
	6	800	801.04	100.13				
FA	1	26.60	26.52	99.69	100.47	1.3774	1.3709	0.0382
	2	26.60	27.13	101.99				
	3	26.60	26.22	98.60				
	4	26.60	27.13	101.99				
	5	26.60	26.83	100.86				
	6	26.60	26.52	99.69				

TABLE-7

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION (AREA UNDER THE CURVE AND SIMULTANEOUS EQUATION METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
IB	1	800	100.14	100.08	0.2157	0.3901	0.2157	0.3883
	2	800	99.74	100.86				
	3	800	100.08	100.49				
Mean			99.98	100.47				
FA	1	26.60	100.50	100.07	0.4041	0.9650	0.4036	0.9557
	2	26.60	100.20	100.08				
	3	26.60	99.70	101.99				
Mean			100.13	100.71				

* Mean of Three Observations

TABLE-8

INTRA DAY AND INTER DAY ANALYSIS OF FORMULATION (DERIVATIVE SPECTRA METHOD)

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
IB	1	800	100.00	101.10	1.4001	0.5818	1.3954	0.5786
	2	800	99.13	99.94				
	3	800	101.87	100.60				
Mean			100.33	100.54				
FA	1	26.60	99.42	100.83	0.6467	1.1844	0.6481	1.1788
	2	26.60	100.53	99.16				
	3	26.60	99.40	101.45				
Mean			99.78	100.48				

* Mean of Three Observations

TABLE – 9

RUGGEDNESS STUDY
(AREA UNDER CURVE AND SIMULTANEOUS EQUATION METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
IB	Analyst 1	99.95	0.7238	0.7241	0.0804
	Analyst 2	100.16	0.7650	0.7637	0.0850
FA	Analyst 1	100.33	1.4000	1.3954	0.1555
	Analyst 2	100.63	0.7234	0.7188	0.0803

TABLE – 10

RUGGEDNESS STUDY
(DERIVATIVE SPECTRA METHOD)

Drug	Condition	Average* % Obtained	S.D	% R.S.D	S.E.
IB	Analyst 1	100.17	0.0321	0.0320	0.0035
	Analyst 2	100.14	0.2230	0.2226	0.0247
FA	Analyst 1	99.88	0.2650	0.2653	0.0294
	Analyst 2	99.40	0.3329	0.3349	0.0369

TABLE-11

RECOVERY STUDIES

(AREA UNDER THE CURVEAND SIMULTANEOUS EQUATION METHOD)

Drug	% Level	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D(+/-)	R.S.D	S.E
IB	80	15	24	39.04	24.04	100.16	0.4366	0.4352	0.0485
	100	15	30	44.99	29.99	99.98	0.4763	0.4710	0.0431
	120	15	36	51.29	36.29	100.81	0.5042	0.5042	0.0462
FA	80	0.5	0.8	1.30	0.80	100.00	0.5499	0.5487	0.0611
	100	0.5	1	1.49	0.99	99.79	0.5921	0.5937	0.0725
	120	0.5	1.2	1.71	1.21	100.83	0.5674	0.5692	0.0682

TABLE-12

RECOVERY STUDIES(DERIVATIVE SPECTROSCOPY METHOD)

Drug	% Level	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered (µg/ml)	% Recovered	S.D(+/-)	R.S.D	S.E
	80	15	24	38.91	23.91	99.62	0.4687	0.4686	0.0520
IB	100	15	30	45.16	30.16	100.53	0.4932	0.4960	0.0492
	120	15	36	50.96	35.96	99.88	0.5120	0.5161	0.0553
	80	0.5	0.8	1.31	0.81	101.87	1.4530	1.4440	0.1614
FA	100	0.5	1	1.49	0.99	99.00	1.3745	1.3767	0.1720
	120	0.5	1.2	1.71	1.21	100.83	1.5181	1.5313	0.1693

TABLE-13

OPTICAL CHARACTERISTICS OF IBUPROFEN AND FAMOTIDINE (RP-HPLC)

PARAMETERS	IBUPROFEN	FAMOTIDINE
λmax	265	265
Beer's law limit (µg/ml)	300-900	10-15
Correlation co-efficient	0.9998	0.9991
Slope (m)	1266.33	21268.74
Intercept (c)	36861.63	177864.60
LOD (µg/ml)	0.0872	0.0366
LOQ (µg/ml)	0.0264	0.0999

TABLE-14

SYSTEM SUITABILITY PARAMETERS FOR THE OPTIMIZED CHROMATOGRAM BY RP-HPLC METHOD

PARAMETERS	IBUPROFEN	FAMOTIDINE
Retention time	10.238	2.400
Tailing factor	1.082	0.795
Asymmetric factor	0.956	0.582
Theoretical plate per unit	56053	1950
Resolution	0.000	40.210

TABLE-15

ASSAY OF TABLET FORMULATION BY RP-HPLC METHOD

Drug	Sample No.	Labelled Amount (mg/tab)	Amount Found (mg/tab)	Percentage Obtained	Percentage Average	S.D (+/-)	% R.S.D	S.E
IB	1	800	788.27	98.53	98.36	0.9343	0.9499	0.0259
	2	800	798.58	99.82				
	3	800	787.55	98.44				
	4	800	778.55	97.31				
	5	800	778.96	97.37				
	6	800	789.60	98.70				
FA	1	26.60	26.01	97.78	96.81	1.5866	1.6388	0.0441
	2	26.60	26.47	99.51				
	3	26.60	25.54	96.01				
	4	26.60	25.23	95.04				
	5	26.60	25.63	96.35				
	6	26.60	25.59	96.20				

TABLE-16

RECOVERY STUDIES OF IBUPROFEN AND FAMOTIDINE BY RP-HPLC METHOD

Drug	% Level	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered	% Recovery	S.D (+/-)	% R.S.D	S.E
IB	80	15	24	37.91	22.91	98.75	0.466	0.471	0.051
	100	15	30	44.82	29.82	99.28	0.121	0.122	0.013
	120	15	36	50.99	35.99	100.17	0.510	0.509	0.056
FA	80	0.5	0.8	1.29	0.79	99.80	0.718	0.719	0.079
	100	0.5	1	1.49	0.99	99.34	1.793	1.805	0.199
	120	0.5	1.2	1.69	1.19	99.47	1.028	1.034	0.114

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